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[Authors]. [Abstract Title]. Program No. XXX.XX. 2014 Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience, 2014. Online.

Nanosymposium

010. Neurogenesis and Neurotransmission in Neurodegenerative Diseases

Location: 152A

Time: Saturday, November 15, 2014, 1:00 PM - 2:45 PM

Presentation Number: 10.01

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

Support: R01 EB008432

R01 NS080655

R01 MH097268

R01 MH085667

P41 EB015922

Title: Capturing brain connectivity in Alzheimer's disease by evolving the human connectome

Authors: *G. PRASAD[1], S. H. JOSHI[2], P. M. THOMPSON[1];
[1]Dept. of Neurology, UCLA Sch. of Med., Imaging Genet. Ctr., Marina Del Rey, CA; [2]Dept. of Neurol., UCLA, Los Angeles, CA

Abstract: Diffusion MRI allows us to represents white matter connectivity as a set of fibers or curves using tractography methods. These fibers are used to quantify the connectivity strength between pairs of anatomical regions from a well known atlas of the cortex to create a connectivity network of the living human brain. In diseases such as Alzheimer's disease (AD), this network configuration can become compromised and we can study the affected connections to gain more insight into the disease. However, the regions from an anatomical atlas may not be optimal for studying disease and we use a method call evolving partitions to improve connectomics (EPIC) to pick the optimal regions to map connectivity in AD. We analyzed 87 participants the Alzheimer's disease Neuroimaging Initiative (ADNI) dataset composed of 37 AD participants and 50 healthy older controls. The participants were scanned using structural and diffusion MRI on a 3-Tesla GE Medical Systems. We computed fiber connectivity in each participant using a global tractography algorithm and a set of cortical regions using the Desikan-Killiany atlas in Freesurfer. The combination of these two results created connectivity networks representing healthy controls and AD. We then used EPIC to evolve the cortical regions such that the differences between controls and AD were maximized as assessed by the accuracy of a machine learning classifier between the two groups (Fig. 1). We treated the standard anatomical

parcellation from Freesurfer as our baseline model and its corresponding accuracy as our benchmark. The cross-validation accuracy using the anatomical parcellation was 82.7% (78.3% specificity, 86% sensitivity). EPIC was able to improve on this accuracy by evolving the parcellation to obtain an accuracy of 85.0% (81.0% specificity, 88% sensitivity). Our results show that we are indeed able to find a better representation of brain connectivity for AD. This new atlas of brain connectivity emphasizes the changes that occur with the onset of dementia and could be a useful alternative to standard connectivity when studying the disease.

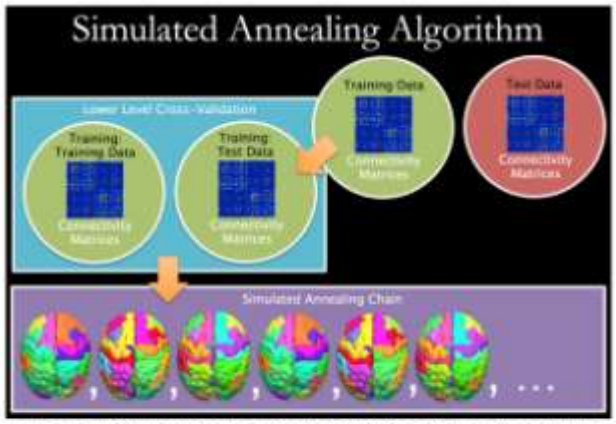

Figure 1. Simulated Annealing to Evolve the Connectome. Our Evolving Partitions to Improve Connectomics (EPIC) method separates connectivity networks in controls and disease into training and testing subsets. It then runs simulated annealing on the training data to evolve the optimal connectivity profile for studying Alzheimer's disease and differentiating it from controls. The resulting optimal connectivity structure could be a preferable way to study the connections in disease by emphasizing the pathways that are most important.

Disclosures: G. Prasad: None. **S.H. Joshi:** None. **P.M. Thompson:** None.

Nanosymposium

010. Neurogenesis and Neurotransmission in Neurodegenerative Diseases

Location: 152A

Time: Saturday, November 15, 2014, 1:00 PM - 2:45 PM

Presentation Number: 10.02

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

Support: Douglas and Ellen Rosenberg Foundation

Joseph Drown Foundation

NIH AG034427

Bredesen Acceleration Project, Sponsored Research Holdings, LLC

Title: The alpha7nAChR partial agonist, 5-HT3 antagonist, and APP-binding re-purposed compound tropisetron increases sAPPalpha, lowers Abeta and p-tau, and normalizes cognition in a murine Alzheimer's model

Authors: *P. SPILMAN[1], O. DESCAMPS[1], O. GOROSTIZA[1], K. POKSAY[1], J. CAMPAGNA[1], A. MATALIS[1], C. PETERS-LIBEU[1], R. RAO[1], V. JOHN[1,2], D. E. BREDESEN[1,2];
[1]Buck Inst. For Age Res., Novato, CA; [2]Univ. of California, Los Angeles, CA

Abstract: It is thought the number of cases of Alzheimer's disease (AD) will rapidly increase in the coming decades. AD is characterized by amyloid-beta (Abeta) plaques and tau neurofibrillary tangles. As by the time of diagnosis substantial pathology is present, it is likely that anti-AD therapy will require chronic, pre-symptomatic treatment; this will make drug safety imperative. In AD there is a shift in amyloid precursor protein (APP) processing toward the anti-trophic state, reducing sAPPalpha relative to sAPPbeta and Abeta species. Restoration of this balance may be essential to reverse the underlying mechanisms that lead to AD. High-throughput screening (HTS) of a clinical compound library in 7W CHO cells expressing huAPPwt was used to identify “hits” increasing sAPPalpha. The hit tropisetron was re-tested in primary neuronal culture, and then determined to be brain-permeable in pharmacokinetic analysis. A series of in vivo studies were performed using 5-6 mo J20 PDAPP Swe/Ind and NTg mice, including a 28-day study (0.5 mg/kg/day) wherein working memory was assessed using the Novel Object Recognition (NOR) task, a 56-day study (0.5 mg/Kg/day) wherein spatial memory was tested by Morris Water Maze (MWM) and 3-month study using 12-20 mo post-plaque J20 and NTg mice treated orally at 4 mg/kg/day; NOR and spatial memory in the Novel Location Recognition (NLR) paradigm were assessed pre-, mid- and end-study. Biochemical analysis included sAPPalpha, sAPPbeta, and phospho-tau (AlphaLISA, Perkin-Elmer) and Abeta 1-40 and 1-42 (ELISA, Life Technologies) of combined hippocampal and entorhinal cortical tissue. IHC analysis included plaque and synaptic load. Tropisetron increased sAPPalpha by 30% in HTS and by 25% in primary culture. In the 28-day study, NOR was increased in tropisetron-treated J20s to NTg levels at 14 days and was even higher than NTg controls at 28 days. sAPPalpha was significantly increased and Abeta 1-40 and 1-42 significantly decreased. In the 56-day study, tropisetron-treated mice showed improvements in MWM. sAPPalpha significantly increased and Abeta 1-42 significantly decreased. Finally, tropisetron improved working and spatial memory at both mid- and end-study in old J20 and NTg mice, and dramatically increased sAPPalpha and decreased phospho-tau. Our iterative hierarchical screening method identified tropisetron which was found to be effective at the human-equivalent doses, improving memory and the biomarkers sAPPalpha, Abeta, and p-tau in brain tissue. As tropisetron has an excellent safety profile - and as a result of the findings here - it is currently in clinical trials for the treatment of mild cognitive impairment (MCI) and early AD.

Disclosures: **P. Spilman:** None. **O. Descamps:** None. **O. Gorostiza:** None. **K. Poksay:** None. **J. Campagna:** None. **A. Matalis:** None. **C. Peters-Libeu:** None. **R. Rao:** None. **V. John:** None. **D.E. Bredesen:** None.

Nanosymposium

010. Neurogenesis and Neurotransmission in Neurodegenerative Diseases

Location: 152A

Time: Saturday, November 15, 2014, 1:00 PM - 2:45 PM

Presentation Number: 10.03

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

Support: German Science Foundation (DFG) Grant PE1193/2-1

DZNE

Title: Purinergic signaling mediates astrocytic hyperactivity *in vivo* in a mouse model of Alzheimer's disease

Authors: A. DELEKATE[1], M. FUECHTEMEIER[2], M. BRUECKNER[3], T. SCHUMACHER[1], A. HALLE[3], *G. C. PETZOLD[1];
[1]German Ctr. For Neurodegenerative Dis. (DZNE), Bonn, Germany; [2]Exptl. Neurol., Charité, Berlin, Germany; [3]Ctr. for Advanced European Studies and Res. (Caesar), Bonn, Germany

Abstract: Astrocytes form dynamic networks in the brain, contribute to cerebrovascular regulation, and support synaptic function. In Alzheimer's disease (AD), astrocytes adopt a "reactive" phenotype, and have been shown to display hyperactivity. The goal of this study was to identify the molecular signaling pathways governing astroglial hyperactivity, and to explore the relationship between astrocytic network dysfunction and reactive astrocytosis as well as cerebrovascular pathology. To this end, we used a standard mouse model of AD (APPPS1 line) to investigate cellular calcium dynamics, cerebral blood flow and Aβ plaque topography in vivo by multiphoton microscopy. We found that significantly more astrocytes were spontaneously hyperactive in APPPS1 mice compared to wildtype age-matched littermates. This hyperactive astroglial phenotype was most pronounced around Aβ plaques, and co-localized with reactive astrogliosis. Moreover, APPPS1 mice displayed astrocytic intercellular wave-like events that propagated across the cortex and in some cases were associated with cerebrovascular changes. Astrocytic hyperactivity remained unchanged when we blocked synaptic transmission or mGluR5-mediated neuron-to-astrocyte signaling. However, inhibition of P2 purinoreceptors strongly reduced hyperactivity. Similarly, inhibition of nucleotide release through connexin

hemichannels also decreased hyperactivity. Inhibition of P2X receptors had no effect, while blockade of IP3 receptor activation downstream of P2Y receptor activation reduced hyperactivity and calcium waves. To better define the involved P2Y receptor subtype, we applied an ectonucleotidase with high ATPase/ADPase ratio, thereby increasing tissue ADP concentration, and found that this treatment strongly amplified hyperactivity and calcium waves, indicating an involvement of ADP-sensitive P2Y receptors. In contrast, all of these interventions, except for IP3R blockade, had no effect on astrocytic transients in wildtype littermates. In conclusion, we have shown that astroglial network dysfunction is mediated by purinergic signaling in reactive astrocytes, and that modulation of P2Y receptor activity or nucleotide release through hemichannels may represent novel targets to ameliorate neuro-glial network dysfunction in AD.

Disclosures: **A. Delekate:** None. **M. Fuechtemeier:** None. **M. Brueckner:** None. **A. Halle:** None. **G.C. Petzold:** None. **T. Schumacher:** None.

Nanosymposium

010. Neurogenesis and Neurotransmission in Neurodegenerative Diseases

Location: 152A

Time: Saturday, November 15, 2014, 1:00 PM - 2:45 PM

Presentation Number: 10.04

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

Support: R01AG033570

RC1 AG036208-01

Title: Depletion of neurogenesis induces cognitive deficits in Alzheimer’s disease

Authors: *C. L. HOLLANDS[1], R. SCHLOESSER[3], K. MARTINOWICH[4], S. KERNIE[5], O. LAZAROV[2];
[2]Anat. and Cell Biol., [1]The Univ. of Illinois at Chicago, Chicago, IL; [3]Univ. of Maryland Med. Ctr., Baltimore, MD; [4]The Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [5]Columbia Univ., New York, NY

Abstract: Alzheimer's disease (AD) is the most prevalent cause of dementia in the elderly. AD is a learning and memory disorder, characterized by progressive loss of memory and cognitive decline. About 95% of AD cases are sporadic, and aging is the greatest risk factor for the disease. The molecular mechanism underlying memory loss is not fully understood. Increasing evidence suggests that impairments in hippocampal neurogenesis take place early in mouse models of

familial Alzheimer's disease. Taken together with the observation that neurogenesis declines with age, this may suggest that impairments in hippocampal neurogenesis play a role in cognitive deficits in AD. To address this hypothesis, we utilized a transgenic approach to temporally regulate depletion of neurogenesis by nestin-regulated expression of δ-HSV-TK in neural progenitor cells in the brain of APPswe/PS1ΔE9 animals. Depletion of neurogenesis was manifested by a 75.7% (p=.004) decrease in nestin expressing cells and a 72% (p=.03) decrease in neuroblasts in the subgranular layer, of the hippocampus. This was matched by an 86% decrease in new neurons in the granular layer as assessed by expression of NeuN and incorporation of 5'-Bromo-2'deoxyuridine (BrdU). Significantly, we observed that the combination of FAD-linked genes and loss of neurogenesis enhanced memory deficits at 4 months of age in the Radial Arm Water Maze test, Pattern Separation and Fear Conditioning tests. These deficits were not observed when neurogenesis was ablated only or when APPswe/PS1DE9 mice were used only. These experiments provide, for the first time, evidence that impairments in neurogenesis promote cognitive deficits in Alzheimer's disease.

Disclosures: C.L. Hollands: None. **R. Schloesser:** None. **K. Martinowich:** None. **S. Kernie:** None. **O. Lazarov:** None.

Nanosymposium

010. Neurogenesis and Neurotransmission in Neurodegenerative Diseases

Location: 152A

Time: Saturday, November 15, 2014, 1:00 PM - 2:45 PM

Presentation Number: 10.05

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

Support: R01AG033570

RC1 AG036208-01

Title: Reduced levels of presenilin-1 in adult hippocampal neural progenitor cells induces learning and memory deficits

Authors: *J. A. BONDS[1], Y. KUTTNER-HIRSHLER[2], N. BARTOLOTTI[2], A. GADADHAR[2], M. PIZZI[3], R. MARR[3], O. LAZAROV[2];
[1]Anat. and Cell Biol., Univ. of Illinois, Chicago, Chicago, IL; [2]Univ. of Illinois at Chicago, Chicago, IL; [3]Rosalind Franklin Univ. of Med. and Sci., Chicago, IL

Abstract: Presenilin-1 (PS1) is the catalytic core of the aspartyl protease γ-secretase, which cleaves numerous membrane proteins, including amyloid precursor protein, notch-1 and other proteins involved in neurogenesis. Mutations in PS1 cause familial Alzheimer's disease (FAD), a progressive neurodegenerative disease characterized by loss of memory and cognitive decline. We previously showed that PS1 regulates neural progenitor cell differentiation in the adult brain. New neurons are thought to play a role in aspects of learning and memory. However, it is not clear if PS1 plays a role in neurogenesis-dependent learning and memory. To determine that, we injected lentivirus expressing small interfering RNA (siRNA) to knockdown PS1 expression in neural progenitor cells in the dentate gyrus of adult mice and evaluated their learning and memory performance 3 months and 6 months following the injection. Here we show that down-regulation of PS1 in hippocampal neural progenitor cells causes progressive deficits in hippocampus-dependent learning and memory function. Furthermore, we demonstrate a decrease in dendritic arborization and spine density in neurons infected with the PS1 lentivirus. These results support a role for neurogenesis in learning and memory and provide a mechanism by which dysfunction of PS1 in Alzheimer's disease compromises learning and memory.

Disclosures: J.A. Bonds: None. **Y. Kuttner-Hirshler:** None. **N. Bartolotti:** None. **A. Gadadhar:** None. **M. Pizzi:** None. **R. Marr:** None. **O. Lazarov:** None.

Nanosymposium

010. Neurogenesis and Neurotransmission in Neurodegenerative Diseases

Location: 152A

Time: Saturday, November 15, 2014, 1:00 PM - 2:45 PM

Presentation Number: 10.06

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

Support: R01AG033570

1RC1AG036208-01

NS20498

Title: Cyclic-AMP Response Element Binding Protein (CREB) signaling is impaired in a mouse model of Alzheimer's disease

Authors: *N. BARTOLOTTI[1], Y.-S. HU[1], D. STORM[2], O. LAZAROV[1];
[1]Univ. of Illinois At Chicago, Chicago, IL; [2]Univ. of Washington, Seattle, WA

Abstract: Alzheimer's disease is a neurodegenerative disorder characterized by cognitive impairment and memory deficits. The phosphorylation of cyclic-AMP Response Element Binding Protein (CREB) is a critical step in the formation of memories. Phosphorylation of CREB on Serine 133 (pCREB-SER^{133}) is necessary for Cyclic-AMP Response Element (CRE)-driven transcription of genes important for learning and memory such as Brain-Derived Neurotrophic Factor (BDNF). Here, we show that steady state levels of pCREB in the APPSwe/PS1E9 mouse model of Alzheimer's disease are reduced compared to levels in the brains of wild type littermates. Notably, following experience in an enriched environment, an experience that increases synaptic plasticity and hippocampal neurogenesis, phosphorylated CREB is increased in the hippocampus of wild-type mice, but not in the hippocampus of APPSwe/PS1e9 mice. Reporter CRE-β-galactosidase/ APPSwe/PS1E9 mice exhibit reduced CRE-gene transcription in the hippocampus compared to CRE-β-galactosidase/wild type littermates. These impairments in CREB signaling are observed prior to the onset of amyloid pathology and inflammation in these mice. Taken together, these experiments suggest that a critical pathway in learning and memory is impaired in FAD-linked APPSwe/PS1E9 mice and may underlie learning and memory impairments exhibited by these mice.

Disclosures: **N. Bartolotti:** None. **Y. Hu:** None. **D. Storm:** None. **O. Lazarov:** None.

Nanosymposium

010. Neurogenesis and Neurotransmission in Neurodegenerative Diseases

Location: 152A

Time: Saturday, November 15, 2014, 1:00 PM - 2:45 PM

Presentation Number: 10.07

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

Support: Zenith Award ZEN-12-241433 from Alzheimer's Association, USA)

Title: Rescue of cognitive deficit with a neurogenic/neurotrophic compound in 3xTg-AD mouse model

Authors: *N. BAAZAOUI[1,2], K. IQBAL[1];
[1]New York Inst. For Basic Res., Staten Island, NY; [2]Biol., CUNY the graduate center, New York, NY

Abstract: Alzheimer's disease (AD) is a devastating neurodegenerative disease and the sixth leading cause of death in the United States. Till now there is no effective drug that can stop or slow the progression of the disease. The most popular approach to treat AD is to inhibit

neurodegeneration. However, another exciting strategy is to treat AD by shifting the balance from neurodegeneration to regeneration of the brain. Here we report the therapeutic beneficial effect of a neurogenic/neurotrophic compound called P021 (Li, B et al. FEBS letters, 2008) on cognitive performance in 3xTg-AD mouse model of AD. At three months of age 3xTg-AD mice were tested for reference memory by Morris Water Maze task and right after they were put on P021 diet. At the age of 12 months these mice were tested again by Morris Water Maze task employing 3 probe trials: the first probe trial was given 24 h after the last training session, the second probe trial 20 days after the first probe trial and the third probe trial 20 days later. At 18 months of age the mice were tested by novel object location test to evaluate short-term spatial reference memory. Then at 20 months of age they were tested for episodic memory by novel object recognition test. The 3 month 3xtg-AD mice showed a clear impairment in reference memory. At 12 months of age the 3xTg-AD mice were found impaired by the Morris Water Maze task during acquisition seen as longer escape latency and less time spent in target quadrant compared to wild type (WT) animals in all the probe trials. The memory impairment was rescued in the P021-treated 3xTg-AD mice. At 18 months of age in the object location test, the 3xTg-AD mice spent more time investigating the familiar location of the object than the novel one, and the treatment with P021 recued this impairment. Using the novel object recognition task at 20 months of age we found that the 3xTg-AD mice spent more time investigating the familiar object than the novel one, and this impairment was rescued in P021 treated animals. All together these data show that chronic treatment with neurogenic/neurotrophic compound can rescue the cognitive impairment in the 3xtg-AD mice.

Disclosures: **N. Baazaoui:** None. **K. Iqbal:** None.

Nanosymposium

012. Demyelinating Disorders

Location: 152B

Time: Saturday, November 15, 2014, 1:00 PM - 4:15 PM

Presentation Number: 12.01

Topic: C.09. Demyelinating Disorders

Support: NMSS RG4078

Title: Neurodegenerative consequences of episodic metabolic stress in oligodendrocytes

Authors: *D. Z. RADECKI[1], A. GOW[2];
[2]CMMG, [1]Wayne State Univ., Detroit, MI

Abstract: In Multiple Sclerosis (MS), the impetus for studying neurodegenerative and non-immune mediated components of the disease is increasing because recent clinical trials and research suggest that autoimmunity may not be the sole, or possibly even primary driver of the pathophysiology. To explore this issue, we developed a novel mouse model to generate MS-like pathology mediated through a primary metabolic stress response in oligodendrocytes. Our model, the *OBiden* (*OBi*) mouse, uses CreER^{T2} recombinase to activate expression of a mutant form of the abundant, myelin proteolipid protein-1 (PLP1), in mature oligodendrocytes of adult mice. This mutant protein induces focal metabolic stress in oligodendrocytes, leading to dysfunction or cell death and subsequent remyelination. Our weekly protocol of metabolic stress induction generates temporal waves of oligodendrocyte pathology and repair, and appears to recapitulate several features of the pathophysiology observed in the most common Relapse-Remitting form of MS, (RRMS). Herein, we focus on the secondary neurodegenerative and behavioral consequences of disease in OBi mice. We initiate pathology at 2 months following baseline MRI, spatial memory and novel object behavioral tests. These tests are repeated at 4 and 6 months of age to assess longitudinal degenerative changes in the mice. At 6 months, we perform several behavioral tests not amenable to longitudinal studies, comprising forced swim, tail suspension, open field and fear conditioning to identify depression-like, anxiety or motivation-dependent memory deficits. Mice are then sacrificed for immunocytochemistry, western blot, qPCR and MRS analyses. At 6 months, MRI reveals that third ventricle volume in *OBi* mice is increased, indicative of periventricular atrophy. White matter changes and apparent focal lesions are also evident. Behaviorally, OBi mice exhibit depression-like behavior compared to controls, as well as deficits in spatial memory. At the molecular level, we observe gross changes in phosphorylated and non-phosphorylated neurofilament proteins throughout cortical and thalamic brain regions, with both unilateral and bilateral presentation. In addition, we observe focal astrocyte scarring and microglial activation consistent with observed pathology in patients with RRMS. Together, these data indicate that the secondary degenerative aspects of disease in *OBi* mice are reminiscent of some features of RRMS patients. Accordingly, these mice will enable us to define molecular mechanisms relevant to MS, particularly the links between oligodendrocyte pathology, neurodegeneration and behavioral and cognitive deficits.

Disclosures: D.Z. Radecki: None. **A. Gow:** None.

Nanosymposium

012. Demyelinating Disorders

Location: 152B

Time: Saturday, November 15, 2014, 1:00 PM - 4:15 PM

Presentation Number: 12.02

Topic: C.09. Demyelinating Disorders

Support: Guthy Jackson Charitable Foundation

NIH/NINDS

German Research Foundation

Title: Effects of neuronyelitis optica autoantibody at the blood-brain barrier

Authors: *Y. TAKESHITA[1,2], B. OBERMEIER[1], A. COTLEUR[1], S. SPAMPINATO[1], Y. SANO[2], T. KANDA[2], R. M. RANSOHOFF[1];
[1]Neurosci., Cleveland Clin. Fndn. Lerner Res. Inst., CLEVELAND, OH; [2]Neurol. and Clin. Neurosci., Yamaguchi Univ. Grad. Sch. of Med., Yamaguchi, Japan

Abstract: [Background] Neuromyelitis optica (NMO), an autoimmune inflammatory astrocytopathy, is caused by antibodies to the astrocyte water channel aquaporin 4 (AQP4). AQP4 is expressed exclusively on astrocytes and absent from endothelial cells. The IgG plasma fraction of NMO patients (NMO-IgG) contains AQP4 antibodies. It remains uncertain how NMO-IgG in plasma accesses and attacks astrocytes across the blood brain barrier (BBB). [Aim] We examined effects of NMO-IgG at vascular (endothelial cells) or intrathecal (astrocytes) side of BBB. [Method] We established in vitro static and flow based BBB models incorporating a conditionally immortalized human brain microvascular endothelial cell line (EC) and human astrocyte cell lines with (A4) or without (A) AQP4 expression. EC and A4 (EC/A4) or A (EC/A) were co-cultured on each side of polycarbonate membranes. After exposing NMO- or control-IgG to vascular or intrathecal side, we evaluated EC activation, IgG accumulation across the EC, barrier function, chemokine expression and leukocyte migration. NMO-IgG was pooled from therapeutic plasma exchange from total 50 NMO patients and control IgG came from healthy subjects' serum. We screened A4 and A pre-exposed to NMO-IgG for cytokines and chemokines. [Results] Vascular application of NMO-IgG activated EC and elevated IgG accumulation in EC/A4 and EC/A. Intrathecal application of NMO-IgG decreased barrier function and increased chemokine expression in EC and leukocyte migration in EC/A4 but not EC/A. Of 20 cytokines, NMO-IgG selectively induced IL-6 in A4 but not A. IL-6 application decreased barrier function and increased chemokine expression in EC and induced leukocyte (especially monocyte) migration. IL-6 receptor blockade inhibited NMO-IgG induced leukocyte migration. [Conclusion] These results indicate that 1) NMO-IgG contains IgGs aside from AQP4 antibodies, which directly modulate EC functions; and 2) NMO-IgG induces IL-6 in astrocytes via AQP4 and Il-6 signaling to ECs decreases barrier function, increases chemokines and drives leukocyte migration.

Disclosures: Y. Takeshita: None. **B. Obermeier:** None. **A. Cotleur:** None. **S. Spampinato:** None. **Y. Sano:** None. **T. Kanda:** None. **R.M. Ransohoff:** None.

Nanosymposium

012. Demyelinating Disorders

Location: 152B

Time: Saturday, November 15, 2014, 1:00 PM - 4:15 PM

Presentation Number: 12.03

Topic: C.09. Demyelinating Disorders

Support: F31 NS084691

R21 NS081598-02

Title: c-kit signals differentially regulate susceptibility to CNS disease in males and female SJL mice

Authors: *A. RUSSI[1,3], M. CAULFIELD[4], M. BROWN[2];
[2]Microbiology-Immunology, [1]Northwestern Univ., Chicago, IL; [3]Med. Scientist Training Program, Feinberg Sch. of Medicine, Northwestern Univ., Chicago, IL; [4]Dept. of Neurol., Mayo Clin., Rochester, MN

Abstract: Females are more susceptible to autoimmune diseases, including multiple sclerosis (MS), a demyelinating disease of the central nervous system. Although genetic, hormonal, and immune differences have been implicated; the specific mechanisms that underlie this female predominance remain unclear. The SJL mouse model of MS, experimental autoimmune encephalomyelitis (EAE), recapitulates many of the features of the human disease; including the relapsing-remitting course and significant sex dimorphism. When immunized with the myelin peptide PLP(139-151), females develop characteristic episodes of severe clinical disability interspersed with pronounced clinical remissions. In contrast males exhibit only mild clinical symptoms, if any. We previously reported that c-kit, the receptor for stem cell factor, regulates disease severity in SJL females through its effects on mast cell development. Mast cell deficient c-kit mutant (Kit-W/Wv) SJL female mice exhibit significantly less severe disease than their wild type (WT) counterparts. Systemic or local meningeal mast cell reconstitution restores WT-like disease parameters. Despite equivalent peripheral myelin-specific T cell responses, immune cell influx into the CNS is compromised in Kit-W/Wv mice, indicating that mast cells control inflammatory cell access to the CNS in females. In contrast, male SJL-Kit-W/Wv mice develop significantly more severe EAE than their WT littermates. This difference in disease severity corresponds to a more robust peripheral CD4+ T cell response during the preclinical phase of disease and is independent of mast cells. Male Kit-W/Wv mice exhibit higher percentages and numbers of encephalitogenic GM-CSF- and IL-17-producing PLP(139-151)-specific CD4+ T cells in their draining lymph nodes when compared to WT controls. Thus c-kit signaling exerts

disparate effects during EAE disease in males and females. In females, c-kit+ mast cells in the meninges are pathogenic and promote inflammatory cell influx into the CNS by compromising the integrity of the blood-brain barrier. However in males, c-kit exerts protective effects in the periphery by limiting the magnitude and controlling the quality of the early autoreactive T cell response. Studies are ongoing to define the relevant c-kit+ cell(s) in males and determine their mechanism of action.

Disclosures: **A. Russi:** None. **M. Caulfield:** None. **M. Brown:** None.

Nanosymposium

012. Demyelinating Disorders

Location: 152B

Time: Saturday, November 15, 2014, 1:00 PM - 4:15 PM

Presentation Number: 12.04

Topic: C.09. Demyelinating Disorders

Support: VA Merit Review

Title: Anti-heterogenous nuclear ribonuclear protein a1 antibodies contribute to neurodegeneration in multiple sclerosis

Authors: ***J. DOUGLAS**[1,3], L. GARDNER[2,3], M. LEVIN[1,4];
[1]Neurol., [2]Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN; [4]Neurol., [3]Veteran Affairs Med. Ctr., Memphis, TN

Abstract: Multiple Sclerosis (MS) is the most common demyelinating disorder of the central nervous system. Neurodegeneration is a dominant feature of progressive forms of MS and a viral model of MS, human T-lymphotrophic virus type-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP). Neurodegenerative mechanisms in these patients are beginning to be uncovered. MS and HAM/TSP patients produce autoantibodies to heterogenous nuclear ribonuclear protein A1 (hnRNPA1). hnRNPA1 is an RNA binding protein highly expressed in human neurons that regulates mRNA transport and metabolism. We hypothesized that anti-hnRNPA1 antibodies might contribute to neurodegeneration by altering nuclear/cytoplasmic trafficking of hnRNP A1 protein. In addition, we hypothesize as a consequence of altered transport, mRNA normally bound to hnRNP A1 for nuclear/cytoplasmic transport and translation would in turn become ineffective resulting in altered functional protein levels of such genes. Our data reveals anti-hnRNPA1 antibodies altered cellular health in a SK-N-SH neuronal cell line by decreasing cellular ATP levels and causing an increase in caspase 3/7 levels resulting in

apoptosis. Also, anti-hnRNPA1 antibodies caused redistribution of endogenous hnRNPA1 protein from a primarily nuclear localization to an equally nuclear and cytoplasmic redistribution. We have also discovered that hnRNPA1 protein interacts with spastin (SPG4) mRNA. SPG4 is a gene, which when mutated results in hereditary spastic paraparesis (HSP), which is a spastic disorder clinically indistinguishable from progressive forms of MS and HAM/TSP. Our data also indicates that anti-hnRNPA1 antibodies cause alterations in protein levels of the spinal paraplegia genes SPG4, SPG7, and SPG20, all of which when mutated result in forms of HSP. Furthermore, in experimental autoimmune encephalomyelitis (EAE), a mouse model for MS, our preliminary results show the addition of anti-hnRNP A1 antibodies worsens clinical symptoms likely resulting in increased neurodegeneration in the brain and spinal cord. Our data suggest that anti-hnRNPA1 antibodies cause deleterious effects in neuronal cells by altering metabolic pathways and localization/expression of endogenous proteins within neurons while also producing damaging effects seen in the EAE mouse model.

Disclosures: J. Douglas: None. **L. Gardner:** None. **M. Levin:** None.

Nanosymposium

012. Demyelinating Disorders

Location: 152B

Time: Saturday, November 15, 2014, 1:00 PM - 4:15 PM

Presentation Number: 12.05

Topic: C.09. Demyelinating Disorders

Support: NYSTEM Grant C026413

NYSTEM Grant C026428

Title: Transcriptional network-based identification of functional human oligodendrocyte differentiation genes

Authors: *S. U. POL[1,3], H. SHAYYA[4], M. O'BARA[1], S. ANDREADIS[2], F. J. SIM[1];
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Abstract: Oligodendrocytes and their myelin are destroyed during demyelinating disease. Oligodendrocyte progenitor cells (OPCs) can restore lost oligodendrocytes and myelin via a regenerative process known as remyelination. In multiple sclerosis, remyelination is limited by impaired OPC differentiation. Thus, the identification of molecular signals that regulate human

OPC differentiation may provide novel means to induce remyelination. In this study, we isolated CD140a$^+$ OPCs from human fetal brain and extracted RNA each day during differentiation *in vitro*. As expected, we observed large up-regulation of MBP mRNA (315 fold, p-value=0.005, n=4). Microarray-based differential expression analysis identified 36 genes significantly regulated during differentiation. Weighted Gene Co-expression Network Analysis (WGCNA) was then applied to define differentiation-specific networks. We tested the functional importance of individual genes within these networks by lentiviral over-expression in human OPCs. At 4 days, over-expression of a novel OPC-specific extracellular component significantly reduced the fraction of differentiating O4$^+$ oligodendrocytes, from 17±5% in mCherry controls to 11±3% (p-value<0.5, n=4). In contrast, over-expression of a specific G-protein subunit expressed during differentiation induced oligodendrocyte differentiation (O4$^+$ 24±7%, p-value<0.05, n=4). To confirm a functional role *in vivo*, human OPCs over-expressing each gene were transplanted into neonatal *shiverer/rag2* mice. Our preliminary data suggest that these genes regulate human OPC differentiation and oligodendrocyte myelination *in vivo*. Thus, the human OPC transcriptional profile accurately predicted pharmacologically-relevant genes that may be targeted to control differentiation.

Disclosures: S.U. Pol: None. **H. Shayya:** None. **M. O'bara:** None. **S. Andreadis:** None. **F.J. Sim:** None.

Nanosymposium

012. Demyelinating Disorders

Location: 152B

Time: Saturday, November 15, 2014, 1:00 PM - 4:15 PM

Presentation Number: 12.06

Topic: C.09. Demyelinating Disorders

Support: NIH GRANT 1R21NS083042-01A1

NTSAD GRANT

Title: Molecular mechanisms of Canavan disease pathogenesis

Authors: *M. TRAKA[1], S. S. SCHERER[2], B. POPKO[1];
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Abstract: Canavan disease (CD) is a rare autosomal recessive leukodystrophy that is caused by mutations of the aspartoacylase gene (ASPA). ASPA is highly expressed in mature

oligodendrocytes, where it catalyzes the hydrolysis of the most abundant amino acid in the brain, N-acetyl-aspartate (NAA) to acetate and aspartic acid. The mechanism of CD pathogenesis, however, remains unknown. One hypothesis is that the loss of ASPA results in reduced levels of acetate, a precursor for myelin lipid synthesis. We recently described the identification of the ENU-induced nonsense mutation, Q193X, in the mouse Aspa gene that results in the absence of detectable ASPA protein expression in Aspanur7 homozygous mutant mice. These mutant mice display severe spongy degeneration of myelin throughout the CNS correlating with increased NAA levels, strikingly resembling CD. Although ASPA's activity peaks at the beginning of the second postnatal week in the mouse brain, the period of active myelination, no clear evidence has yet been found concerning its role in this process. We investigated if ASPA function is important for myelination in the CNS using both in vitro and mouse genetics approaches. We established pure oligodendrocyte cultures from early postnatal Aspanur7 mutant mice to detect potential differentiation and survival defects in the ASPA-deficient oligodendrocytes. Our data indicate that these cells are able to differentiate in vitro to mature myelin-forming cells that showed normal survival and expressed abundant levels of all the major myelin proteins. We also used oligodendrocyte cell cultures to demonstrate that high levels of N-acetylaspartate (NAA) and the NAA glutamate derivative (NAAG) detected in the CSF of the CD patients has no toxic effect on differentiating wild-type and Aspanur7 mutant oligodendrocytes. We are currently investigating whether ASPA is required for supporting formation or maintenance of the myelin once oligodendrocyte processes ensheath and wrap around the axons by establishing myelinating cocultures of retinal ganglion neurons with oligodendrocyte progenitor cells. Furthermore, we generated myelin deficient Aspanur7 mutant mice by crossing them to MBP mutant mice, shiverer (MBPshiv). The Aspanur7/nur7;MBPshiv/shiv double mutant mice displayed a severe neurological phenotype and early lethality that correlates with increased vacuolation throughout the CNS. Therefore, the ASPA deficiency has a negative effect on mutant oligodendrocytes that produce no compact myelin, indicating that ASPA has a potential role outside myelination. This finding further suggests that impaired myelination is not likely the leading cause of CD pathology.

Disclosures: **M. Traka:** None. **S.S. Scherer:** None. **B. Popko:** None.

Nanosymposium

012. Demyelinating Disorders

Location: 152B

Time: Saturday, November 15, 2014, 1:00 PM - 4:15 PM

Presentation Number: 12.07

Topic: C.09. Demyelinating Disorders

Support: NIH R37NS041435 to P.A.C.

NIH R21 NS081418-02

NMSS Collaborative Center Grant

The Silverman Foundation

Title: Assessing remyelination in an inflammatory environment: A novel adoptive transfer cuprizone model

Authors: *E. G. BAXI, D. TOSI, L. KIRBY, J. DEBRUIN, I. GRISHKAN, A. FAIRCHILD, P. CALABRESI, A. GOCKE;
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Abstract: The identification of therapeutics that promote remyelination might be useful in the treatment of multiple sclerosis (MS). While spontaneous remyelination is efficient in many acute primary demyelinating diseases, remyelination efficiency declines and fails in chronic demyelinating diseases such as MS, likely for a number of reasons. Our lab has focused on chronic CNS inflammation as one important mechanism that may hinder efficient remyelination in MS. While EAE has been useful to study immune cell activation and CNS trafficking, there is significant axonal pathology, which limits the potential to study mechanisms of endogenous remyelination. Cuprizone mediated demyelination is useful for studying demyelination and endogenous remyelination, but there is less inflammation to study the mechanisms by which immune cells may inhibit myelin repair. Therefore, a need exists for a model that more closely mimics both the CNS inflammation and primary demyelination aspects of MS. Here, we describe a new MS model in which we adoptively transfer polarized myelin reactive effector T-cells (TEFF) into 4 week cuprizone fed mice at the time of dietary cuprizone withdrawal. We demonstrate that transferred TEFF are able to infiltrate into the CNS where they recruit host immune cells. We further show that the adoptive transfer of the Th17 cell subtype results in a higher population of CD4+ cells in the brain. Immune cell infiltration was correlated with a significant decrease in CNPase, MBP and MAG protein levels. Importantly, the extent of reduction in myelin associated protein is dependent, at least in part, by the efficiency of immune cell infiltration into the brain. Ultrastructural examination by electron microscopy of corpus callosal tissue revealed a significant decrease in the number of myelinated axons in AT-cuprizone animals, while the overall axon number remained equivalent across all groups. Taken together, these results suggest that our AT-cuprizone model is characterized by pronounced focal primary demyelination with reduced endogenous remyelination and minimal axonopathy in the presence of inflammation. The AT-Cuprizone model could be used to study the immune related mechanisms by which remyelination fails and to develop therapeutic strategies that may help to

overcome this blockade, which may more accurately recapitulate what likely occurs in MS than do our present models of remyelination.

Disclosures: E.G. Baxi: None. **D. Tosi:** None. **L. Kirby:** None. **J. DeBruin:** None. **I. Grishkan:** None. **A. Fairchild:** None. **P. Calabresi:** None. **A. Gocke:** None.

Nanosymposium

012. Demyelinating Disorders

Location: 152B

Time: Saturday, November 15, 2014, 1:00 PM - 4:15 PM

Presentation Number: 12.08

Topic: C.09. Demyelinating Disorders

Support: board of directors, Tisch MS Research center of NY

Title: Metabolomics of cerebrospinal fluid reveals differential signatures of progressive multiple sclerosis

Authors: *F. MIR, D. LEE, H. RAY, S. A. SADIQ;
TISCH MS RESEARCH CENTER OF NY, NY, NY

Abstract: The purpose of the current study was to determine and compare the global metabolic profiles in human cerebrospinal fluid (CSF) associated with disease progression in multiple sclerosis (MS) and allow stratification of the two progressive disease subtypes. CSF obtained from controls with no disease (n=15), patients with primary progressive multiple sclerosis (PPMS; n=15), and patients with secondary progressive multiple sclerosis (SPMS; n=15) were analyzed on the GC/MS and LC/MS/MS platforms conducted by Metabolon (Durham, NC). A total of 198 compounds of known identity were included in the analysis. The data obtained revealed 26 biochemicals that were significantly altered in the MS cohort as compared to the controls. Interestingly, the analysis also revealed differences between the primary progressive and secondary progressive populations with significant differences in 18 biochemicals. Of particular interest are the changes in carbohydrate metabolism, creatine and creatinine metabolism, extracellular matrix (ECM) remodeling and neuroactive amino acids. One of the most consistent changes in the dataset included accumulation of metabolites related to carbohydrate metabolism, including glucose utilization through glycolysis and the pentose phosphate pathway (PPP), in CSF from patients with PPMS or SPMS. In particular, increased levels of the biochemical indicator of glucose utilization, lactate, as well as elevations in several pentose sugars that may be obtained from the diet or synthesized through the PPP (ribulose,

ribitol, xylonate, xylose, xylitol, arabinose, and threitol) were noted in both MS patient groups, with slightly more pronounced changes in patients with SPMS as compared to PPMS. Moreover, higher levels of metabolites involved in alternative pathways of glucose utilization, such as sorbitol and fructose, along with increased levels of the sugar alcohol mannitol and its derivative mannose were also observed in the MS groups. These changes in carbohydrate metabolism may be related to a host of metabolic changes in brain tissue of patients with PPMS or SPMS ranging from energy generation to maintenance of glutathione to combat oxidative stress. In contrast, elevations in pro-hydroxy-pro, a biochemical marker of ECM remodeling, was found to be more pronounced in the PPMS than in the SPMS group, as compared to the controls. In conclusion, results from this global profiling study revealed perturbations in the CSF metabolome that were both consistent and different when comparing patients with PPMS or SPMS and will aid in our understanding of progressive disease mechanisms in MS.

Disclosures: F. Mir: None. **D. lee:** None. **H. ray:** None. **S.A. sadiq:** None.

Nanosymposium

012. Demyelinating Disorders

Location: 152B

Time: Saturday, November 15, 2014, 1:00 PM - 4:15 PM

Presentation Number: 12.09

Topic: C.09. Demyelinating Disorders

Support: NMSS Pilot grant to Lidia Gardner

Department of Veterans Afairs Merit Review to Michael Levin

Title: Low Apolipoprotein A1 levels have profound effects on MS pathogenesis

Authors: *L. A. GARDNER[1,2], J. N. DOUGLAS[2], M. C. LEVIN[2,1];
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Abstract: Apolipoproteins play important roles in cholesterol transfer and lipid metabolism in the central nervous system. Among six major classes of Apolipoproteins (A, B, C, D, E and H) only apolipoprotein E (ApoE) has been studied extensively in neurobiology. The importance of Apolipoprotein A1 (ApoA1) has been identified in atherosclerosis, cardiovascular and cognitive diseases, however its function in multiple sclerosis (MS) has not been fully investigated. ApoA1 is the most abundant component of high-density lipoprotein (HDL). HDL-associated ApoA1 may play a role in neuronal regeneration by acting as a constitutive anti-inflammatory factor. We

identified differential ApoA1 expression in serum and plasma of MS patients and healthy controls. MS patients with progressive disease exhibited decreased ApoA1 levels in their plasma. Low ApoA1 levels had inverse correlation with Expanded Disability status scale (EDSS). ELISA data was confirmed with quantitative Western analysis. Further, we investigated the role of ApoA1 in a mouse model of MS. ApoA1 deficient female mice (C57Bl/6-Tg(ApoA1)1Rub/J) demonstrated higher incidence and severity of the experimental autoimmune encephalomyelitis (EAE) in comparison to the wild type control mice (C57Bl/6J). EAE was accompanied by increase in cytokines (INF-, TNF-, TGF-ß, IL-2, IL-23) and T cell differentiation into CD25+/Foxp3+ T cells in these animals. In addition to immunological parameters we measured neurological function of the optical nerve with visual Evoked Potentials. (VEPs). ApoA1 deficient mice experienced greater loss of optic nerve function in comparison to control animals with EAE. Neurodegeneration (ND) and demyelination (DM) was scored on histological brain and spinal cord sections stained with either FluroJade C or luxol fast blue (LFB). ApoA1 deficient mice on average had 1.5 times higher DND and DM scores compared to C57Bl/6J EAE mice and 4 times higher scores compared to naïve animals. Taken together our data suggests that low levels of ApoA1 have profound effects on immunological and neurological systems in immune medicated neurological disease such as multiple sclerosis. Further investigation into the mechanisms of ApoA1 formation and disease-associated loss might lead to novel therapies in MS.

Disclosures: L.A. Gardner: None. **J.N. Douglas:** None. **M.C. Levin:** None.

Nanosymposium

012. Demyelinating Disorders

Location: 152B

Time: Saturday, November 15, 2014, 1:00 PM - 4:15 PM

Presentation Number: 12.10

Topic: C.09. Demyelinating Disorders

Title: TGM6 is a potential biomarker in MS and its expression by reactive astrocytes in the murine spinal cord during EAE correlates with disease course

Authors: *M. CRISTOFANILLI, D. GRATCH, B. PAGANO, D. BATES, S. A. SADIQ; Tisch MS Res. Ctr. of New York, NEW YORK, NY

Abstract: Transglutaminase 6 (TGM6) is a member of the transglutaminase enzyme family found predominantly in the central nervous system, and is expressed mainly by neuronal cells under physiological conditions. TGM6 has been proposed as the autoimmune target in gluten-

sensitive patients with neurological symptoms such as cerebellar ataxia and has been linked to other neurological conditions such as schizophrenia. Its relative transglutaminase 2 is the primary auto-antigen in celiac disease and has been found to exacerbate MOG-induced experimental autoimmune encephalomyelitis (EAE) in mice through positive regulation of T cell differentiation and inflammation. Here we investigate the involvement of TGM6 protein and antibodies raised against it in multiple sclerosis (MS) and EAE. Using enzyme-linked immunosorbent assays (ELISA), we found that antibodies raised against TGM6 are elevated in MS patient CSF compared to controls. In addition, our data shows that TGM6 protein and antibody against it are elevated in the CSF of primary progressive MS compared to the CSF of control individuals, relapsing remitting and secondary progressive MS patients. To further investigate if changes inTGM6 expression might be pathogenic in vivo, we employed the EAE animal model, which primarily affects the spinal cord via demyelination and astrogliosis. Using ELISA and western blot, we found that mouse TGM6 protein levels are increased at disease onset and correlate with its course. Furthermore, immunostaining of mouse spinal cord at disease peak revealed strong expression of TGM6 in reactive astrocytes, which was not found in control animals. In conclusion, in this study we provide evidence that CSF levels of TGM6 protein and antibodies against it could be useful biomarkers to: diagnose MS vs. other neurological conditions; differentiate between MS subtypes; and predict disease activity and progression. In addition, the finding that TGM6 is strongly expressed in reactive astrocytes during EAE suggests a potential role for this protein in the mechanism of glial scar formation in MS.

Disclosures: **M. Cristofanilli:** None. **S.A. Sadiq:** None. **D. Gratch:** None. **B. Pagano:** None. **D. Bates:** None.

Nanosymposium

012. Demyelinating Disorders

Location: 152B

Time: Saturday, November 15, 2014, 1:00 PM - 4:15 PM

Presentation Number: 12.11

Topic: C.09. Demyelinating Disorders

Support: National Multiple Sclerosis Society (NMSS) Grant

Title: Combining magnetization transfer ratio MRI and quantitative measures of walking improves the identification of fallers in MS

Authors: ***N. E. FRITZ**[1], R. E. R. MARASIGAN[2], J. KELLER[2], C. CHIANG[2], C. K. JONES[3], A. ELOYAN[4], P. A. CALABRESI[5], K. M. ZACKOWSKI[6];

[1]Motion Analysis Lab., Kennedy Krieger Institute, Johns Hopkins Universit, Baltimore, MD; [2]Motion Analysis Lab., Kennedy Krieger Inst., Baltimore, MD; [3]Radiology and Radiological Sci., [4]Biostatistics, [5]Neurol., Johns Hopkins Univ., Baltimore, MD; [6]Motion Analysis Lab., Kennedy Krieger Institute, Johns Hopkins Univ., Baltimore, MD

Abstract: Background: Multiple sclerosis (MS) is a progressive demyelinating disease affecting the central nervous system. Balance and walking are frequently impaired in individuals with MS, resulting in accidental falls and injury. Falls in MS have been associated with higher Expanded Disability Status Scale (EDSS) scores and poor balance. Magnetic resonance imaging (MRI) is a common clinical tool for monitoring disease progression. An exploration of infratentorial lesions in individuals with known balance dysfunction showed that falls were positively associated with lesion volume in the middle cerebellar peduncle and brainstem. However, the relationship of walking and falls to tract specific MRI measures and quantitative clinical measures of strength and sensation has not been explored in MS. Objective: To examine the relationship and predictive value of clinical measures and tract specific brain MRI measures sensitive to myelin (magnetization transfer ratio (MTR)) and axonal integrity (fractional anisotropy (FA)) in the corticospinal tract (CST) to quantitative measures of walking and fall status (faller v. non-faller). Methods: 23 individuals with relapsing-remitting MS (mean ± SD age: 49.1 ± 11.5 years; 12 females; EDSS: 3.9 ± 1.5; symptom duration: 14.1 ± 10.2 years; 10 fallers) participated in a 3T brain MRI including diffusion tensor imaging (DTI) and MTR, as well as clinical tests of walking, strength, sensation and a falls history. Region of interest selection for the CST was performed in DTIStudio. Spearman Correlation and Forward Stepwise Logistic Regression were used to assess the relationships of walking and MRI measures on fall status. Results: Walking velocity, Timed Up and Go (TUG) and Timed 25 Foot Walk performance were significantly associated with CST FA (r=-0.4879; p=0.0248; r=-0.6117; p=0.0054; r=0.5447; p=0.0107, respectively) and MTR (r=-0.4331; p=0.0441; r=-0.4744; p=0.0257; r=0.4704; p=0.0272, respectively). Interestingly, a model including CST MTR and TUG explained >47% of the variance in fall status (R2=0.4739; MTR p-value=0.071; TUG p-value=0.040) and accurately distinguished fallers from non-fallers with a cross-validation error of 23%. Inclusion of DTI, strength and sensation measures did not improve the model. Conclusion: These preliminary results suggest that tract specific MTR captures may be useful in relating brain pathology to walking performance and fall status in MS. This data is part of an ongoing study; thus, additional subjects and brain volume MRI variables will be added which may improve identification of significant associations between MRI measures and clinical measures of walking and falls in MS.

Disclosures: **N.E. Fritz:** None. **R.E.R. Marasigan:** None. **J. Keller:** None. **C. Chiang:** None. **C.K. Jones:** None. **A. Eloyan:** None. **P.A. Calabresi:** F. Consulting Fees (e.g., advisory boards); Vaccinex, Vertex, Abbott, MedImmune, Prothena. **K.M. Zackowski:** None.

Nanosymposium

012. Demyelinating Disorders

Location: 152B

Time: Saturday, November 15, 2014, 1:00 PM - 4:15 PM

Presentation Number: 12.12

Topic: C.09. Demyelinating Disorders

Support: MSCRFII-0193-00=90043101

Title: Hypercellularity within white matter of dysmyelinated and immunodeficient shiverer mice is reversed following myelination by transplanted glial progenitor cells and that process can be monitored *in vivo* by MRI

Authors: *A. ARNOLD[1,2], J. ZHANG[1,2], A. JABLONSKA[1,2], S. SAJJA[1], M. JANOWSKI[1,3,4], P. WALCZAK[1,5];
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Abstract: A wide range of neurological disorders result in loss or dysfunction of myelin. Transplantation of glial restricted progenitors (GRPs) capable of differentiating towards myelinating oligodendrocytes may be an effective approach to restore brain function in patients with myelin disorders. Non-invasive imaging of remyelination is important for monitoring efficacy of cell therapy; however, it remains a challenging task. Various processes are known to interfere with MR imaging of myelin, including edema or inflammation. We observed that cell density within white matter of dysmyelinated shiverer mice is significantly higher than in wild type mice. In MRI it is reflected by hyperintensity in T2, low MTR and low anisotropy in DTI. The goal of this study was to assess whether GRP transplantation leads to myelination detectable by MRI and if myelination affects the white matter hypercellularity. Human fetal GRPs (Q Therapeutics®) or mouse GRPs were transplanted bilaterally into the lateral ventricles of neonatal rag2-/- shiverer mice (P1-3) and followed with MRI and histology. MRI of grafted and control mice was performed serially using Bruker 11.7T scanner (T2, MTR, DTI). Animals were sacrificed at 360, 440, and >500 days post transplantation (DPT) for assessment of grafted cells with immunohistochemistry and DAPI to evaluate cell density within white matter. Shiverers engrafted with mGRPs survived max. 200 days, this corresponded with the life expectancy of non-transplanted controls. Histological analysis revealed the restricted engraftment and myelination within the periventricular corpus callosum at 120 DPT. The myelinated regions were

detected by MRI. In contrast, lifespan of mice grafted with hGRPs was dramatically extended with 57% of grafted mice surviving over 400 days. MBP staining revealed extensive engraftment, migration and myelination at 420 DPT. At the same time point MRI data for the white matter continued to appear hyperintense in T2, with low MTR and FA suggesting no myelination. After 450 DPT, the MRI scans for hGRP grafted shiverers showed pattern that was consistent with myelination including hypointense corpus callosum in T2, high MTR and FA. Calculation of cell density within white matter (DAPI) revealed that detection of myelin in MRI coincided with significant drop of cell density. Decrease of cell density was confirmed for human and mGRP transplants and it spatially overlapped with engraftment and myelination by GRPs. In conclusion, transplanted hGRPs showed therapeutic potential superior to mGRPs. Myelination of white matter in shiverer mice leads to reduced cellularity and that appears to be required for detection of myelin in MRI.

Disclosures: **A. Arnold:** None. **J. Zhang:** None. **A. Jablonska:** None. **M. Janowski:** None. **P. Walczak:** None. **S. Sajja:** None.

Nanosymposium

012. Demyelinating Disorders

Location: 152B

Time: Saturday, November 15, 2014, 1:00 PM - 4:15 PM

Presentation Number: 12.13

Topic: C.09. Demyelinating Disorders

Support: NMSS

Title: Alternatively activated macrophage signaling in CNS remyelination

Authors: ***J. K. HUANG**, K. PSACHOULIA, K. A. CHAMBERLAIN, S. E. NANESCU; Biol., Georgetown Univ., Washington, DC

Abstract: Impaired CNS myelin regeneration (remyelination) in late stage multiple sclerosis (MS) results in progressive neurodegeneration. The reason for remyelination failure is unknown, but one plausible explanation is that environmental signals necessary for oligodendrocyte precursor cell (OPC) differentiation are inappropriately regulated or diminished in chronic lesions. Here we analyzed a temporally resolved transcriptional profile of spontaneous CNS remyelination, and identified an unsuspected immunomodulatory enzyme called interleukin-4 induced gene 1 (IL4i1) in remyelinating lesions. IL4i1 encodes a secreted L-amino acid oxidase and has been mapped to a chromosomal locus of autoimmune disease susceptibility, including

MS. However its role in CNS injury/repair has not previously been suggested. We found that IL4i1 is strongly upregulated during remyelination following mouse spinal cord demyelination, and is expressed by alternatively activated, anti-inflammatory (M2) microglia/macrophages. Moreover, we found that IL4i1 gain-of-function significantly increased oligodendrocytes and myelin basic protein expression in lesions. We suggest that IL4i1 positively regulates CNS remyelination and that targeting IL4i1 has therapeutic potential in myelin repair.

Disclosures: J.K. Huang: None. **K. Psachoulia:** None. **K.A. Chamberlain:** None. **S.E. Nanescu:** None.

Nanosymposium

013. Psychomotor Stimulant Reinforcement

Location: 140A

Time: Saturday, November 15, 2014, 1:00 PM - 3:30 PM

Presentation Number: 13.01

Topic: C.17. Drugs of Abuse and Addiction

Title: Drug-seeking behavior in ants: A new model for morphine-induced reward, self-administration, and addiction

Authors: *B. V. ENTLER[1], J. CANNON[2], M. A. SEID[3];
[1]Neurosci., [2]Psychology, [3]Biol., The Univ. of Scranton, Scranton, PA

Abstract: Conventional definitions of drug abuse and addiction are primarily focused on characterizing human neurophysiological and behavioral responses. Although animal models, such as those of mammals, have been proven invaluable in studying specific and complex aspects of addiction, invertebrate systems have proven advantageous in investigating how drugs of abuse corrupt the most basic motivational and behavioral systems. Using a sucrose-fading paradigm followed by a two-dish preference test, this study establishes the ant species *Camponotus floridanus* as a new model for opiate self-administration and drug-seeking behavior. When compared to controls (Naïve and Sucrose-conditions), Morphine-conditioned ants exhibit a complete inversion in their preference of natural rewards (sucrose) to morphine. Remarkably, prior to this study only mammalian animal systems demonstrated active drug seeking without the concurrent presence of a natural reward. Our experiment consisted of three different exposure conditions: Naïve, Sucrose, and Morphine. Drug-Naïve ants tested whether or not a natural preference for morphine existed prior to establishing addiction. The Naïve-condition occurred for only one 4-hour standard two-dish choice test between pure 0.2mg/*mL (aq)* morphine and a 0.5M *(aq)* sucrose. Morphine-conditioned ants experienced morphine exposure for 6-days while

simultaneously experiencing a sucrose-fading paradigm. On day 7, Morphine-conditioned ants experienced the standard two-dish design as outlined above. The choice-test was used to observe whether an addiction to the drug was present, regardless of the absence of caloric value. Sucrose-conditioned ants were rewarded with aqueous sucrose for 6-days while experiencing a sucrose-fading paradigm identical to the Morphine-condition above. On day 7, Sucrose-conditioned ants experienced the two-dish design outlined above, but tested the preference between pure H20 (0.0 M sucrose) and 0.5 M *(aq)* sucrose. The Sucrose-condition tested whether or not the sucrose-fading paradigm produces any changes in the natural rewarding properties of sucrose. Our results indicate that unlike *Drosophila,* which requires the presence of a natural reward, confounding addiction and self-administration, ants will actively seek morphine even in the absence of natural reward and caloric value, i.e. sucrose. Consequently, *C. floridanus* is the first non-mammalian model of addiction and reward known to actively seek a feeder containing a drug of abuse without the presence of a natural reward.

Disclosures: B.V. Entler: None. **J. Cannon:** None. **M.A. Seid:** None.

Nanosymposium

013. Psychomotor Stimulant Reinforcement

Location: 140A

Time: Saturday, November 15, 2014, 1:00 PM - 3:30 PM

Presentation Number: 13.02

Topic: F.03. Motivation and Emotion

Support: NIH Grant DA035443

NIH Grant DA029035

Hellman Foundation Fellowship

UCLA Faculty Career Development award

Title: The role of phasic striatal dopamine signaling in reward seeking and motivation

Authors: *K. M. WASSUM[1], V. Y. GREENFIELD[1], A. L. COLLINS[1], K. E. LINKER[1], S. B. OSTLUND[2];
[1]Psychology, UCLA, Los Angeles, CA; [2]Univ. California, Irvine, Irvine, CA

Abstract: On a daily-basis we make hundreds of reward-related decisions. Considerable evidence suggests that such decisions are goal-directed, that is controlled by the anticipated

incentive value of the potential rewarding goal; individuals consider each decision's outcome before making the choice, often choosing paths leading to more desirable outcomes. Reward-paired cues and contexts also acquire a motivational value and provide an additional invigorating source of motivation for instrumental reward-seeking actions modulating the immediate impact of need state on reward-seeking behavior. Considerable research efforts have focused on determining the neural mechanisms of instrumental learning and decision-making, and have strongly implicated the striatum and dopaminergic signaling therein. Here I present data collected from a series of experiments wherein we used fast-scan cyclic voltammetry to make measurements of dopamine in rats freely behaving in tasks designed to evaluate the role of striatal dopamine signaling in instrumental learning and the use of both goal and cue value to guide such reward-seeking behavior. We have found that nucleus accumbens core dopamine displays properties of a reward prediction error-like signal during self-paced instrumental learning, backpropagating from the unexpectedly earned reward early in training through the sequence of actions that earn the reward coming to precede the initiation of the most distal action in the sequence and eventually backpropagating away from the action itself to cues signaling session onset. Moreover, we find that in the nucleus accumbens core phasic dopamine release relates to the general motivational value provided by reward-paired cues and contexts, but not to specific information provided by behavioral goals. Though it is often assumed that dopamine cells broadcast a unitary signal throughout the striatum, recent subregion-specific phasic dopamine recordings suggest this may not be the case. I will also present data evaluating potential functional dissociations between ventral and dorsal striatal dopamine signaling in reward-seeking motivation.

Disclosures: K.M. Wassum: None. **V.Y. Greenfield:** None. **A.L. Collins:** None. **K.E. Linker:** None. **S.B. Ostlund:** None.

Nanosymposium

013. Psychomotor Stimulant Reinforcement

Location: 140A

Time: Saturday, November 15, 2014, 1:00 PM - 3:30 PM

Presentation Number: 13.03

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Intramural Research Program

Title: Methamphetamine self-administration produces differential effects on the striatal expression of stress-related genes and histone deacetylases

Authors: *J. L. CADET, B. LADENHEIM, M. T. MCCOY, I. KRASNOVA;
Mol. Neuropsychiat Br., NIH/NIDA, Baltimore, MD

Abstract: Methamphetamine use disorder is a chronic neuropsychiatric disorder that is characterized by recurrent binge episodes, interval of cessation of drug taking, and craving-induced relapses to drug use. Rats that accelerate their intake over time will stop their drug-taking behaviors in response to punishment characterized by increasing intensity of foot shocks. In the present study, rats were trained to self-administer methamphetamine for 9 hours over 14 days. One group of rats then received foot-shock during 50% of methamphetamine infusions over 9-10 days, whereas another group was not punished. The rats were euthanized 30 days later and their striata were dissected for the measurements of stress neuropeptides. Expression of several histone deacetylases (HDACs) was also measured. We found significant increases in CRH expression in the rats that had self-administer methamphetamine regardless of shock status. CRHR1 receptor mRNA levels were increased only in shock-treated rats whereas CRHR2 expression was not affected in any of the group. Vasopressin (AVP) was not affected in any group whereas AVPR1a expression was increased in both methamphetamine groups. Interestedly, cocaine and amphetamine-regulated transcript (CART) mRNA levels were increased only in the shock-treated methamphetamine group. Gene expression is regulated, in part, by changes in the abundance of histones acetylated at lysine residues, a process regulated by histone acetyl transferases (HATs) and HDACs. We thus measured the expression of classes I and II HDACs in the rat striatum. HDAC1 and 2 mRNA levels in the methamphetamine groups were not different from controls but the punished rats showed significantly higher expression than the non-punished group. HDAC3 expression was decreased significantly in both methamphetamine groups. HDAC4 expression in the methamphetamine groups was not different from controls. HDAC5 was increased in the shock-treated methamphetamine animals in comparison to control and methamphetamine alone groups. There were no significant methamphetamine-induced changes in striatal HDAC6 expression in comparison to controls. Altogether, these results show that methamphetamine self-administration is associated with altered regulation of a diversity of genes that might impact learning, memory, and synaptic functions. Better understanding of the way that gene products interact to cause methamphetamine addiction will help to develop better pharmacological treatment of methamphetamine addicts.

Disclosures: J.L. Cadet: None. **B. Ladenheim:** None. **M.T. McCoy:** None. **I. Krasnova:** None.

Nanosymposium

013. Psychomotor Stimulant Reinforcement

Location: 140A

Time: Saturday, November 15, 2014, 1:00 PM - 3:30 PM

Presentation Number: 13.04

Topic: C.17. Drugs of Abuse and Addiction

Support: R01 DA019666

University of Minnesota MnDRIVE

Title: *In vivo* opiate administration drives bidirectional and cell-type specific AMPAR plasticity the nucleus accumbens

Authors: *M. C. HEARING[1], J. P. JEDYNAK[2], S. R. EBNER[1], R. A. FISCHER[1], M. J. THOMAS[1];
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Abstract: Plasticity of glutamatergic synapses in the nucleus accumbens (NAc) is driven by exposure to in vivo cocaine and appears to contribute to increases in drug-seeking behavior. However, relatively little is known about whether, how or when experience with other abused drugs may trigger synaptic plasticity in this key node in reward circuits. In this study, we identify adaptations in glutamatergic signaling within the NAc following withdrawal from exposure to repeated morphine. Whole-cell voltage-clamp recordings were performed in medium spiny neurons (MSNs) in sagittal brain slices containing the NAc core or shell regions from adult C57BL/J6 and Drd1a-tdTomato mice. Mice received 5 daily injections of morphine (10 mg/kg, i.p.) or saline, followed by 10-14 d of withdrawal, at which point animals were left undisturbed (no challenge) or injected with saline or morphine 24 h before electrophysiological study (challenge). Initial studies in NAc shell MSNs of wild-type mice showed a significant increase in the ratio of AMPAR:NMDAR currents, a reduction in paired-pulse ratios, and an increase in miniature excitatory postsynaptic current (mEPSC) amplitude, but not frequency in morphine-treated mice following withdrawal (no challenge). In contrast, preliminary data from the core suggest that withdrawal from morphine does not significantly alter any of these parameters. Studies using Drd1a-tdTomato mice show that within the shell, AMPAR/NMDAR ratios, as well as mEPSC amplitude and frequency are increased in D1- but not in non-D1-MSNs (i.e., D2-MSNs), while a significant reduction in frequency was found in D2-MSNs of the core. In morphine-challenged mice, data indicate that a single re-exposure to morphine during withdrawal becomes a potent stimulus for synaptic depression and reverses the initially observed potentiation within the shell. Our results indicate that in vivo morphine exerts dynamic bidirectional control over excitatory synaptic strength in NAc that displays neuronal and anatomical selectivity. Mechanisms underlying this phenomenon and the role morphine-induced depotentiation induced by re-exposure during abstinence may serve to promote the reinstatement of drug-seeking behavior are currently being explored.

Disclosures: **M.C. Hearing:** None. **S.R. Ebner:** None. **R.A. Fischer:** None. **M.J. Thomas:** None. **J.P. Jedynak:** None.

Nanosymposium

013. Psychomotor Stimulant Reinforcement

Location: 140A

Time: Saturday, November 15, 2014, 1:00 PM - 3:30 PM

Presentation Number: 13.05

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA025606

NIH Grant AA013852

Title: Self-administration of both ethanol and methamphetamine increases motivation for methamphetamine

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Abstract: Combined abuse of methamphetamine (METH) and alcohol increases the likelihood of METH-induced psychopathology, and alcohol drinking is a contributing risk factor for discontinuation of drug treatment programs by METH abusers. Consequently, we designed an animal model to investigate the effects of alcohol drinking on motivation for METH, using rats trained to self-administer METH and alcohol in distinct contexts. Rats were trained to press a lever for 10% v/v alcohol in the presence of discriminative olfactory/auditory cue combinations signaling availability (S+ cues) or non-availability (S- cues) of reinforcement. After twenty 30-min training sessions, rats underwent catheter implantation surgery for METH intravenous self-administration (IVSA) procedures. Following recovery from surgery, the rats continued alcohol S+/S- training and initiated METH (0.05 mg/kg per reinforcement) IVSA training in 2-hr (ShA) sessions for 10 days. During these sessions, METH deliveries were paired with the presentation of 3-sec light/tone cues that were distinct from the alcohol-paired cues. The rats were tested for IVSA breakpoints using a 2-day progressive ratio procedure. They then continued alcohol and METH training for 10 more days, using 6-hr (LgA) METH IVSA sessions. After a second 2-day progressive ratio test for METH, the rats underwent extinction training in the IVSA chambers where operant responding had no programmed consequences. After responding decreased to a threshold level, the rats were reintroduced to the ethanol chambers for two 30-min S+ and S-

sessions, where each session was followed by a METH (1 mg/kg, i.p.)-primed reinstatement test in the IVSA chambers. The ethanol-trained rats exhibited greater levels of drug-primed METH-seeking behavior following S+ (drinking) sessions, relative to their METH-seeking behavior after S- (nondrinking) sessions. These rats also demonstrated a significantly higher breakpoints for METH in the progressive ratio test after LgA IVSA training than after ShA IVSA training. Neither of these effects were evident in control groups of rats whose training and testing procedures were identical, except that alcohol was replaced with water in the S+/S- sessions. Additionally, alcohol training did not appear to exert effects on the acquisition rates for METH IVSA or extinction, nor did they change the rats' performance during cue-elicited reinstatement tests in the IVSA chambers. These results suggest that rats trained to self-administer both alcohol and METH exhibited a specific tendency for greater motivation for METH following sessions of alcohol drinking.

Disclosures: P.R. Kufahl: None. **S.B. Taylor:** None. **L.R. Watterson:** None. **N.E. Nemirovsky:** None. **B. Burrows:** None. **M. Olive:** None.

Nanosymposium

013. Psychomotor Stimulant Reinforcement

Location: 140A

Time: Saturday, November 15, 2014, 1:00 PM - 3:30 PM

Presentation Number: 13.06

Topic: C.17. Drugs of Abuse and Addiction

Title: The role of αCaMKII in the establishment of cocaine's reinforcing effects

Authors: *C. P. MUELLER[1], A. LOURDUSAMY[2], M. HAVRANEK[3], K. MIZUNO[4], J. SOLATI[6], Y. GOLUB[6], T.-K. CLARKE[7], H. VALLADA[8], R. LARANJEIRA[9], S. DESRIVIERES[5], G. H. MOLL[6], R. MÖSSNER[10], J. KORNHUBER[1], G. SCHUMANN[5], K. P. GIESE[4], C. FERNANDES[5], B. QUEDNOW[3], A. C. EASTON[5];
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Abstract: Cocaine is a widely used illicit psychostimulant drug with a considerable addiction potential. Despite this, the means of establishing cocaine-use associated behaviors are uncertain but believed to involve molecular mechanisms of learning and memory. Alpha-Ca^{2+}/calmodulin-dependent protein kinase-II (αCaMKII) is a key mediator of learning and memory also involved in drug-related plasticity. The autophosphorylation of αCaMKII was shown to accelerate learning. Here, we investigated the role of αCaMKII autophosphorylation in the time course of establishing cocaine addiction-related behavior in mice. We found that αCaMKII autophosphorylation deficient $\alpha CaMKII^{T286A}$ mice show delayed establishment of conditioned place preference, but no changes in acute behavioral activation, sensitization, or conditioned hyperlocomotion to cocaine. In-vivo microdialysis revealed that $\alpha CaMKII^{T286A}$ mice showed delayed dopamine (DA) and blocked serotonin (5-HT) responses in the nucleus accumbens (NAcc) and prefrontal cortex after acute cocaine administration. Under cocaine, the attenuated DA and 5-HT activation in $\alpha CaMKII^{T286A}$ mice was followed by reduced c-Fos activation in the NAcc. In order to translate the rodent findings to human conditions, several CAMK2A gene polymorphisms were tested regarding their risk for a fast establishment of high cocaine consumption in two independent samples of regular cocaine users from Brazil (n=688) and Switzerland (n=141). A meta-analysis across both samples confirmed that carriers of a CAMK2A single nucleotide polymorphism display a faster transition to severe cocaine use. Together, these data may suggest that αCaMKII and its autophosphorylation controls the speed of how cocaine-use associated behaviors are established.

Disclosures: C.P. Mueller: None. **J. Kornhuber:** None. **A. Lourdusamy:** None. **M. Havranek:** None. **K. Mizuno:** None. **J. Solati:** None. **Y. Golub:** None. **T. Clarke:** None. **H. Vallada:** None. **R. Laranjeira:** None. **S. Desrivieres:** None. **G.H. Moll:** None. **R. Mössner:** None. **G. Schumann:** None. **K.P. Giese:** None. **C. Fernandes:** None. **B. Quednow:** None. **A.C. Easton:** None.

Nanosymposium

013. Psychomotor Stimulant Reinforcement

Location: 140A

Time: Saturday, November 15, 2014, 1:00 PM - 3:30 PM

Presentation Number: 13.07

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH F32 DA036326-02

NIH K99/R00-DA024762

NIH R01-DA036582

Title: Viral-mediated transfer of DREADDs reveals a differential role of the corticostriatal pathway in cocaine-taking and cocaine-seeking behavior

Authors: *K. A. KERSTETTER[1], R. STEWART[2], J. F. NEUMAIER[3,4], S. M. FERGUSON[1,3]; [1]CIBR, Seattle Children's Res. Inst., Seattle, WA; [3]Dept. of Psychiatry and Behavioral Sci., [4]Dept. of Pharmacol., [2]Univ. of Washington, Seattle, WA

Abstract: Motivation for drug intake and relapse to drug seeking are two behavioral components of drug addiction that can be modeled in rats using cocaine self-administration. Previous studies have shown that anterior cingulate cortex (ACC) and nucleus accumbens (NA) are involved in regulating motivation for cocaine, but the precise role of ACC afferents to NA in these behaviors are not yet clear. To address this, we utilized a Cre recombinase-dependent viral vector based flip-excision (FLEX) switch system to express DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) selectively in neurons of the ACC that project to the NA of male Long Evans rats. AAV FLEX constructs containing an inverted form of the Gi/o, Gs, or Gq DREADD receptor (hM4Di, hM4Ds, hM3Dq respectively) were infused into the ACC and a CAV Cre-recombinase viral vector (CAV-Cre) was infused into the NA of the same rats. The CAV is retrogradely transported to neuronal cell bodies; thus, only neurons that project from the ACC to the NA express DREADDs. Activation of DREADDs was produced by the otherwise inert ligand clozapine-N-oxide (CNO, 3 mg/kg, i.p.). Following viral expression, rats were trained to self-administer cocaine (.75/mg/kg/inf) and then switched to a PR schedule of cocaine self-administration for testing. Compared to vehicle, CNO treatment significantly increased break points in rats that expressed hM4Di but had no effect in rats that expressed hM4Ds. Preliminary data from rats expressing hM3Dq suggests that CNO treatment decreases break points relative to vehicle. Following PR testing rats underwent extinction of operant behavior followed by cocaine primed (10 mg/kg, ip) reinstatement testing. Compared to vehicle, CNO significantly decreased responding on the lever that previously delivered cocaine in rats expressing hM4Di, but had no effect in the other DREADD groups. Thus, increasing Gi/o signaling in ACC afferents to NA has a different impact on drug behaviors that depends on the phase of drug use (i.e., during drug intake (PR), increasing Gi/o signaling enhances motivation for cocaine whereas the same manipulation following three weeks of abstinence leads to decreased cocaine-seeking behavior). These findings suggest that diminished cortical control may contribute to on-going drug use in addicts whereas drug withdrawal may lead to augmented activity of the cortex during exposure to drug-related stimuli that facilitates relapse. Unexpectedly, these findings also suggest that G-protein dependent signaling cascades in ACC

afferents to NA play distinct roles in drug-taking and drug-seeking behaviors. Funding: NIH F32 DA036326, K99/R00-DA024762, and R01-DA036582.

Disclosures: K.A. Kerstetter: None. **R. Stewart:** None. **J.F. Neumaier:** None. **S.M. Ferguson:** None.

Nanosymposium

013. Psychomotor Stimulant Reinforcement

Location: 140A

Time: Saturday, November 15, 2014, 1:00 PM - 3:30 PM

Presentation Number: 13.08

Topic: C.17. Drugs of Abuse and Addiction

Support: Grant-in-Aid for Young Scientists B 25860392

Grant-in-Aid for Scientific Research C 24530914

Title: Involvement of immune protein mhc class I in the development of cocaine self-administration

Authors: *G. MURAKAMI[1], H. MENG[2], M. EDAMURA[3], T. FURUKAWA[3], A. FUKUDA[3], T. IWASHITA[4], Y. ISHIDA[5], D. NAKAHARA[6];
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Abstract: Cocaine addiction is a psychiatric disorder that remains a serious public health problem worldwide. Because effective pharmacological drugs have not been identified, further understanding of molecular mechanisms behind the development of cocaine addiction is important to find novel targets for the pharmacological treatment. Although transgenic mice are useful for this purpose, it has been technically challenging in mice to establish self-administration paradigm daily for 24 hrs, which mimics human drug use. In our previous study, we succeeded in ensuring daily 24-hr access to cocaine for mice by employing an intracranial drug delivery system with reverse microdialysis technique. Using this novel system, we screened for several knockout mice available at our university, and found that functional deficit of major histocompatibility complex class 1 (MHCI), a major player in the adaptive immune system,

exaggerated cocaine-seeking behavior. It has been recently discovered that MHCI is expressed in the brain that has long been designated as an immune privilege region and plays an important role in the modulation of synaptic plasticity such as elimination of long-term depression and enhanced long-term potentiation, resulting in the enhanced synaptic connections. We sought to evaluate the possibility whether modulation of MHCI expression in the brain is involved in the process of persistent cocaine-seeking behavior of wild type mice after the period of extinction. To this end, we measured mRNAs of MHCI in brain regions involved in the process of cocaine addiction. After subjecting wild-type mice to cocaine self-administration paradigm, we discovered that expression of MHCI was reduced only in the VTA, implying a possibility that MHCI expressed in the VTA is important in the development of cocaine self-administration. In consistent with this idea, cocaine-induced enhancement of synaptic inputs into dopaminergic neurons of the VTA was significantly larger in functional MHCI knockout mice than in wild-type mice, as shown by more increased AMPA/NMDA ratios and amplitudes of mEPSCs by whole cell patch clamp recording. These results indicate that cocaine self-administration leads to reduction of MHCI expression and enhances inputs into dopaminergic neurons in the VTA, resulting in the development of cocaine self-administration.

Disclosures: G. Murakami: None. **H. Meng:** None. **M. Edamura:** None. **T. Furukawa:** None. **A. Fukuda:** None. **T. Iwashita:** None. **Y. Ishida:** None. **D. Nakahara:** None.

Nanosymposium

013. Psychomotor Stimulant Reinforcement

Location: 140A

Time: Saturday, November 15, 2014, 1:00 PM - 3:30 PM

Presentation Number: 13.09

Topic: C.17. Drugs of Abuse and Addiction

Support: NICHD R01HD069238

Title: Effects of poly drug use on serotonin and dopamine pathways in fetal brain

Authors: *M. E. SELZER, G. TATEVOSIAN, N. MERABOVA, N. DARBINIAN, L. GOETZL;
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Abstract: Introduction: The neurotoxic effects of amphetamine, alcohol and opioids use in adult experimental animals include damage to serotonergic and dopaminergic terminals, and changes in dopamine, serotonin and glutamate levels. Prenatal exposure to amphetamines can lead to

structural abnormalities in brain, such as decreased volume of the hippocampus, striatum, and globus pallidus, but the underlying molecular mechanisms are still unclear. Prenatal exposure to amphetamines is often combined with other psychoactive substances use that may change the susceptibility of the developing brain to this potentially damaging effect. As part of our research on the impact of the prenatal exposure of psychoactive substances on fetal brain we are studying the effects of maternal use of Adderall combined with other psychoactive substances on the brain development.
Methods: We administered a questionnaire to women undergoing elective pregnancy termination (2^{nd} trimester) who were classified as "exposed" or "control", based on self-reported amphetamine exposure since conception. We identified those exposed to Adderall (n=6). Four were also exposed to alcohol, opioids (2), antidepressants (2), tobacco (6) and marijuana (3). Control group matched by gestational age, fetal gender and absence of history of alcohol or psychoactive substances use (n=4). HPLCS analysis was performed to confirm amphetamine exposure. Genders of the fetuses were determined by PCR. Expression of 84 genes in the serotonergic and dopaminergic pathways was analyzed by quantitative PCR in fetal brain tissue, comparing exposed and gestational age- and fetal gender-matched controls. Synaptosomal levels of SERT were analyzed by Western blot. Genes examined included those for: Dopamine and serotonin receptors and transporters; dopamine and serotonin synthesis and degradation; signal transduction pathways including PI3/AKT pathway; and key dopamine and serotonin gene targets.
Results: We found changes in expression of genes involved in signal transduction pathways; PI3 kinase (ρ=0.48), Akt isoform 3 (ρ=0.45), MAPK1 (ρ=0.47) and Phosphodiesterase 10A (ρ=0.34) transcripts were up-regulated in exposed groups compared to control groups.
Conclusion: Our data show that fetal exposure to amphetamine results in abnormal gene expression for serotonin and dopamine synthesis, signaling and transport. This data will be confirmed by studies of protein expression and activities in brain, synaptosomes and placenta. Controls will be matched also by maternal race and BMI. Further we will increase the number of subjects and explore dose-dependent responses based on level of amphetamine exposure.

Disclosures: **M.E. Selzer:** None. **G. Tatevosian:** None. **N. Merabova:** None. **N. Darbinian:** None. **L. Goetzl:** None.

Nanosymposium

013. Psychomotor Stimulant Reinforcement

Location: 140A

Time: Saturday, November 15, 2014, 1:00 PM - 3:30 PM

Presentation Number: 13.10

Topic: C.17. Drugs of Abuse and Addiction

Support: University of Florida McKnight Brain Institute

Title: Functional mapping of the central actions of the powerful bath salt MDPV and its effects on resting state brain activity

Authors: *M. FEBO, K. TRAN, L. COLON-PEREZ, K. BLUM, B. A. GOLDBERGER, A. W. BRUIJNZEEL, B. SETLOW, M. S. GOLD;
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Abstract: Synthetic cathinones represent an emergent hazard to public health. These are widely known as 'bath salts' or 'legal highs'. Bath salts are potent stimulant and hallucinogenic drugs, and their abuse has the potential to impair mental health. The various chemical constituents of bath salts share molecular features, biochemical actions, and behavioral effects with a range of other illegal stimulants such as cocaine, methamphetamine and methylenedioxymethamphetamine. Among the bath salts 3,4-methylenedioxypyrovalerone (MDPV) has been reported to exert powerful cocaine-like effects in rats (Baumann et al., Neuropsychopharmacology, 2012). An intriguing behavioral effect often cited by users is a strong urge and craving to use accompanied by euphoria, heightened sociability, emphathy that occurs from 15 minutes to 2-4 hrs following intake. Starting at 6-8 hrs after drug intake users may experience a "crash" characterized by a strong negative affective state with delirium, depression, and in some reported cases suicidal thoughts and violent aggressive behavior. This suggests unconventional CNS actions that may extend beyond the mesolimbic dopamine reward system. Despite the growing number of studies reporting the stimulant and reinforcing actions of bath salts there is still a knowledge gap with regard to their sites of action within the CNS and their effects on functional connectivity between brain regions. The present study was designed (1) to investigate the acute pharmacological actions of a moderate dose of MDPV (0.3 mg/kg, iv) on BOLD activation across a number of corticostriatal, mesolimbic, frontal cortical and limbic subcortical areas, and (2) to examine the effects of MDPV on resting state BOLD activity 1hour after treatment. Rats were imaged at 4.7T at the Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) facility of the University of Florida. We observed that MDPV exerted stronger actions than cocaine in subcortical networks that included striatal regions, accumbens shell, olfactory structures, insular cortex, infralimbic cortex, lateral hypothalamus, and bed nucleus of the stria terminalis. These structures play key roles in emotionality and physiological activation that could underlie the above-mentioned emotional and cognitive effects. In addition, MDPV increased resting state connectivity within the anterior cingulate cortex and between this region and others, and increased interhemispheric connectivity within the accumbens. These preliminary findings warrant attention to, and further investigation of, the potential unconventional actions of cathinone drugs, which may impact long-term mental health.

Disclosures: **K. Tran:** None. **M. Febo:** None. **L. Colon-Perez:** None. **K. Blum:** None. **B.A. Goldberger:** None. **M.S. Gold:** None. **A.W. Bruijnzeel:** None. **B. Setlow:** None.

Nanosymposium

014. Plasticity in the Olfactory System

Location: 147A

Time: Saturday, November 15, 2014, 1:00 PM - 3:30 PM

Presentation Number: 14.01

Topic: B.08. Synaptic Plasticity

Support: Human Frontier Science Program Long-Term Fellowship

NINDS Intramural Program 1ZIANS003002

Title: Rapid and continuous activity-dependent plasticity of sensory input to the mouse olfactory bulb *in vivo*

Authors: *C. E. CHEETHAM[1], L. BELLUSCIO[2];
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Abstract: Olfactory bulb (OB) glomeruli are the initial sites for processing of odor information in the brain, and are thought to represent functional units of odor coding. Olfactory sensory neurons (OSNs) transmit odor information from the nose to the glomeruli of the ipsilateral OB: here, OSN axons synapse with dendrites of both projection neurons and local interneurons. It is well established that OSNs regenerate throughout life, but it has been unclear whether immature OSNs are capable of synapse formation. Furthermore, whether OSN axons and the synapses that they form are stable or dynamic during the 1-2 month lifespan of the neuron is completely unknown. To address these questions, we used the tetracycline transactivator system to express cytosolic tdTomato and GFP-tagged synaptophysin (sypGFP, a presynaptic marker) specifically in either immature OSNs (using the G-gamma8 promoter) or mature OSNs (using the olfactory marker protein promoter). Using immunogold transmission electron microscopy, we first determined that immature OSNs can indeed form functional synapses. We then implanted cranial windows over the OB of juvenile (P21) and adult (P56) mice, and used two-photon imaging to follow the structural dynamics of OSN axons and the presynaptic terminals that they form in real time in vivo. We focused our analyses on the glomerular layer, and excluded highly mobile sypGFP clusters, which are likely to be transport packets. Turnover of presynaptic terminals was evident on a timescale of hours, and was 3-fold higher for immature than for mature axons over 3 hours. However, there was no difference in turnover rate between juvenile and adult mice.

Unilateral naris occlusion in young adult mice resulted in a 3-fold reduction in the turnover of presynaptic terminals for both immature and mature axons in the ipsilateral OB. Our findings demonstrate rapid and continuous activity-dependent turnover of sensory input to the OB even in adult mice, providing an important anatomical substrate for lifelong experience-dependent plasticity. Furthermore, our data suggest that the developmental state of the neuron, rather than the age of the mouse, determines the degree of structural plasticity exhibited by OSN axons.

Disclosures: C.E. Cheetham: None. **L. Belluscio:** None.

Nanosymposium

014. Plasticity in the Olfactory System

Location: 147A

Time: Saturday, November 15, 2014, 1:00 PM - 3:30 PM

Presentation Number: 14.02

Topic: B.08. Synaptic Plasticity

Support: National Basic Research Program (2013CB531304, 2011CB504405)

Natural Science Foundation China (30990261 and 81171033)

Title: Cortical glutamatergic and GABAergic neurons learn to encode odor signal through their coordinated plasticity

Authors: *J. H. WANG[1], Z. GAO[2], D. WANG[2];
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Abstract: Associative learning and memory is essential for the cognitions. Neural plasticity in mammals is presumably cellular mechanism underlying information storage. Neural plasticity may be used to interpret behavioral changes after memory formation, but not signify primary cellular processes for information storage and retrieval. Neural plasticity in previous reports occurs after the cortices receive the quantitative changes of their innate signals, but not the new featured information. The storage of the new information may require the recruitment of the unused neurons and the reformation of the neurons that have stored a signal. How the neurons are recruited and reformed to encode associative signals (innate and newly learnt signals) for their storage and retrieval remains elusive *in vivo.* How the different neurons coordinately encode the new information for storage and retrieval needs to be addressed. By creating a mouse model of associative learning and memory, in which pairing whisker and olfactory stimuli leads to odorant-induced whisker motion, we have investigated these issues in the barrel cortices by

two-photon cell imaging, electrophysiology and microRNA analysis. After associative learning, the substantial portions of barrel cortical excitatory and inhibitory neurons are recruited to encode the new odor signal besides the innate whisker signal. Some of these neurons showed different spatial and temporal activity patterns in response to innate whisker signal and acquired odor signal, and some of them demonstrated similar patterns in their responses. The associative activation of the sensory cortices makes their neurons being able to store the innate signal and the newly learnt signal as well. The neurons of producing different codes in response to the associative signals may distinguish the differences of their characteristics, whereas the neurons of producing similar codes may signify their historical association. Moreover, the functional recruitments of these cortical neurons are accompanied by the upregulation of excitatory subcellular compartments, the downregulation of inhibitory subcellular compartments and the homeostasis of their mutual innervations. Therefore the excitatory and inhibitory neurons in the sensory cortices undergo the coordinated refinement to encode the storage and retrieval of newly learnt signal and innate signal. [This study is granted by National Basic Research Program (2013CB531304, 2011CB504405) and Natural Science Foundation China (30990261 and 81171033) to JHW].

Disclosures: **J.H. Wang:** None. **Z. Gao:** None. **D. Wang:** None.

Nanosymposium

014. Plasticity in the Olfactory System

Location: 147A

Time: Saturday, November 15, 2014, 1:00 PM - 3:30 PM

Presentation Number: 14.03

Topic: B.08. Synaptic Plasticity

Support: Spanish Ministry of Economy and Competitiveness BFU2012-32512,

Generalitat Valenciana ACOMP/ 2012/229

Prometeo Excellence Program PROMETEO2013/069

Fundación Alicia Koplowitz

University of Valencia PhD Grant

Title: Characterization of PSA-NCAM cells in mouse piriform cortex

Authors: *M. B. ESTELLER, A. RUBIO, R. GONZÁLEZ-MARTÍNEZ, I. FARIÑAS, J. NACHER;
Univ. of Valencia, Burjassot, Spain

Abstract: New neurons in the adult brain transiently express molecules related to neuronal development, such as the polysialylated form of neural cell adhesion molecule (PSA-NCAM) or doublecortin (DCX). These molecules are also expressed by a cell population in the layer II of the cerebral cortex of different mammalian species, although their whose origin, phenotype, and function are not clearly understood. The present study analyzes these parameters in the mouse piriform cortex. We analyzed the proportions of the main cellular populations present in the piriform layer II, ie. glial cells, mature and immature neurons. As previously described in rats, most PSA-NCAM expressing cells could be classified as tangled cells and a small proportion as semilunar-pyramidal transitional neurons. Combining an anti-PSA-NCAM antibody with different lineage markers we found that most PSA-NCAM expressing cells did not co-express markers of glial cells or mature neurons. They were mainly immunoreactive for DCX confirming its immature nature. We analyzed the proliferative activity of the adult piriform cortex layer II and demonstrated that in this region very few cells were cycling and none of them was PSA-NCAM positive. The time of origin of the different cell types in the adult piriform cortex layer II was also evaluated using thymidine analog pulse-chase experiments, in which the thymidine analog was administered during embryonic development or during adulthood. We found that cells in this paleocortical region, including PSA-NCAM positive cells, were born during embryonic development and failed to identify any PSA-NCAM cell generated during adulthood. In addition to these experiments, we have developed in vitro strategies directed to isolate and expand the immature neurons in the adult piriform cortex layer II.

Disclosures: M.B. Esteller: None. **A. Rubio:** None. **R. González-Martínez:** None. **I. Fariñas:** None. **J. Nacher:** None.

Nanosymposium

014. Plasticity in the Olfactory System

Location: 147A

Time: Saturday, November 15, 2014, 1:00 PM - 3:30 PM

Presentation Number: 14.04

Topic: B.02. Ligand-Gated Ion Channels

Title: Deletion of olfactomedin 2 results in abnormal behavior and changes in the composition of the AMPAR complex in mice

Authors: A. SULTANA[1], N. NAKAYA[1], L. DONG[2], *S. I. TOMAREV[1];
[1]SRGCB, LRCMB, [2]GEF, Natl. Eye Institute, NIH, Bethesda, MD

Abstract: Olfactomedin 2 (Olfm2) is a secretory glycoprotein belonging to the family of olfactomedin domain-containing proteins. *OLFM2* has been reported to be associated with primary open angle glaucoma in Japanese patients. Previous studies have identified Olfm1, Olfm2 and Olfm3 proteins as components of the AMPA receptor complex. The main goal of this study was to elucidate possible functions of Olfm2 using *Olfm2* null mice. *Olfm2* null mice were produced by replacing the *Olfm2* gene with the β-galactosidase gene (LacZ) (Olfm2-KO^{LacZ}). Expression of *Olfm2* was detected in the eye and various brain regions using LacZ staining. Olfm1 and Olfm2 proteins co-segregated with the GluR1-4 subunits of the AMPA receptor and other synaptic proteins in the synaptosomal membrane fraction (LP1) upon biochemical fractionation of adult mice cortex. Moreover, Olfm2 physically interacted with the GluR2 subunit. The LP1 fraction of *Olfm2* null mice showed a significant increase in Olfm1, GluR2 and GluR3 proteins as compared with wild-type littermates. The LP1 fraction was used for immunoprecipitation using GluR2 antibody. *Olfm2* null samples showed reduced interaction of GluR2 with some components of the AMPAR complex including CNIH2, PSD95 and Olfm1 as compared with wild-type samples. Analysis of the synaptic vesicle fraction demonstrated a decrease in the levels of caveolin 1 and clatherin heavy chain in *Olfm2* null mice compared with wild-type littermates. *Olfm2* null mice showed reduced exploration, locomotion, olfactory sensitivity, abnormal motor coordination, and anxiety related behavior. Analysis of the functional integrity of the visual pathways using the visual evoked potential test demonstrated that the amplitude of the first negative wave was reduced in *Olfm2* null mice as compared with wild-type littermates. In conclusion, our data suggest that Olfm2 is an important player at the synaptic membrane and is involved in the regulation of the AMPAR composition. Elimination of Olfm2 results in changes in the components of the AMPAR complex and other synaptic proteins leading to changes in the synaptic plasticity and behavior abnormalities.

Disclosures: A. Sultana: None. **N. Nakaya:** None. **L. Dong:** None. **S.I. Tomarev:** None.

Nanosymposium

014. Plasticity in the Olfactory System

Location: 147A

Time: Saturday, November 15, 2014, 1:00 PM - 3:30 PM

Presentation Number: 14.05

Topic: D.01. Chemical Senses

Support: NIH R01 GM086456

NSF IOS 0920024

HFSP RGY0042/2010-C

Title: Regulated olfaction drives state-dependent plasticity in *C. elegans* behavioral prioritization

Authors: *D. S. PORTMAN[1], D. A. RYAN[1], R. M. MILLER[1], K. LEE[2], P. SENGUPTA[3], S. J. NEAL[3];
[1]Ctr. for Neural Develop. and Dis., Univ. of Rochester, Rochester, NY; [2]City Col. of New York, New York, NY; [3]Brandeis Univ., Waltham, MA

Abstract: Recent studies in multiple systems have shown that peripheral sensory function is modulated by cues that encode internal state. However, the mechanisms that underlie this remain poorly understood, as do the roles of regulated sensory function in behavioral flexibility. In the nematode *C. elegans*, we have found that regulated olfactory sensitivity is a key component of the mechanism that generates state-dependent plasticity in the choice between feeding and mate-searching. In animals that prioritize exploration (specifically, well-fed adult males), the attraction to food-derived odorants is low. However, when feeding is prioritized—in juvenile males, in starved adult males, or in hermaphrodites—attraction to these odorants is high. This regulated sensitivity occurs at least in part at the level of sensory neurons themselves, through regulated expression of at least one specific chemoreceptor, ODR-10. In well-fed adult males, the expression of ODR-10 is low; however, food-deprivation or genetic feminization increases ODR-10 expression and food sensitivity. We are currently working to identify the molecular mechanisms that link these three dimensions of internal state—genetic sex, developmental stage, and feeding status—to regulated odorant receptor expression. Using a candidate-based approach, we have found that mutations in several conserved genes disrupt aspects of this regulation, identifying pathways that could generate sensory plasticity in more complex systems. Moreover, our results demonstrate that genetic sex has a key role in sensory neurons and can influence behavior through direct modulation of sensory properties.

Disclosures: D.S. Portman: None. **D.A. Ryan:** None. **R.M. Miller:** None. **K. Lee:** None. **P. Sengupta:** None. **S.J. Neal:** None.

Nanosymposium

014. Plasticity in the Olfactory System

Location: 147A

Time: Saturday, November 15, 2014, 1:00 PM - 3:30 PM

Presentation Number: 14.06

Topic: A.04. Stem Cells

Support: NIH Grant DC012567

Title: Odorant receptor expression in aged mice following genetically-mediated lesion

Authors: *J. H. BRANN[1], S. J. FIRESTEIN[2];
[1]Dept. Biol., Loyola Univ. Chicago, Chicago, IL; [2]Depts. Biol. Sci. and Neurosci., Columbia Univ., New York, NY

Abstract: Several repositories of neuronal stem cells are resident in the nervous system. One population is that found in the peripheral olfactory system, and is capable of generating excitatory projection neurons that extend a long axon from the neuroepithelium lining the nasal cavity to the olfactory bulb. The potential of these basal neuronal stem cells to generate sensory neurons in the young adult epithelium has been known for more than 30 years, but the ability of their counterparts in aged tissue to recapitulate the epithelium is relatively unexplored. We have previously established the regenerative capacity of stem cells in the vomeronasal epithelium is not diminished with age, and have extended our work to the olfactory epithelium as well. However, the gene expression profile of odorant receptors varies with age, such that receptor genes are turned on and off at different ages during the lifetime of the animal. Here we probe the ability of the neuronal stem cell in aged animals to generate the diverse array of neurons expressing the appropriate repertoire of odorant receptors. To this end we generated a line of mice, iDTR x OMP-cre, whereby a Cre-mediated excision of a STOP cassette renders mature neurons sensitive to diphtheria toxin (DT) via activation of the DT receptor. This method permits a specific, sensitive, and reversible ablation of mature (OMP-expressing) neurons upon DT administration but without damage to potential synaptic targets in the OB or to other cell types found in the olfactory epithelium. We administered either DT or saline to adult male mice of several age groups (2, 6, 12, 18 months of age) for six days. Thirty days following ablation, to allow for complete degeneration and subsequent recovery of olfactory epithelia, RNAs were harvested and prepared for microarray analysis. Preliminary results reveal, that when examining the cohort of odorant receptor genes expressed following recovery from lesion, those regenerated at young ages do not significantly differ from those regenerated in aged animals. These results imply that the regenerative potential of the neuronal stem cell in aged animals is intact and is capable of generating a wide array of sensory neurons. However, these results do not explain the observed age-dependent effect on odorant receptor expression observed in normal, intact mice.

Disclosures: J.H. Brann: None. **S.J. Firestein:** None.

Nanosymposium

014. Plasticity in the Olfactory System

Location: 147A

Time: Saturday, November 15, 2014, 1:00 PM - 3:30 PM

Presentation Number: 14.07

Topic: D.01. Chemical Senses

Support: NIH NIDCD R01 DC00644

NIH NIDCD 1F32DC012718-01

NIH NIDCD ROI DC012718

Title: Functional mapping of glomerular inhibition in the olfactory bulb of the awake and anesthetized mouse

Authors: *M. N. ECONOMO[1,2,3], K. R. HANSEN[4], T. BOZZA[5], M. WACHOWIAK[2,3]; [1]Janelia Farm Res. Campus, Ashburn, VA; [2]Brain Inst., [3]Dept. of Neurobio. and Anat., [4]Dept. of Bioengineering, Univ. of Utah, Salt Lake City, UT; [5]Dept. of Neurobio., Northwestern Univ., Evanston, IL

Abstract: Lateral inhibition between functional processing units increases the selectivity of neurons to physical stimuli in many sensory areas. In these systems, this canonical circuit motif is thought to enhance contrast between similar stimuli. In the olfactory system, however, the functional organization of lateral inhibition between glomeruli - the processing units of the olfactory bulb (OB) - is unclear, and how this inhibition maps to odor identity is unknown. Here, targeting an ultra-sensitive fluorescent reporter of neural activity (GCaMP6f) to OB projection neurons, we found that glomerular inhibition can be directly observed using two-photon imaging from the apical dendritic tufts of these cells. Using this approach, we mapped odorant-evoked inhibition in specific glomeruli and characterized its functional properties in awake and anesthetized mice. Single odorants evoked inhibition that was selective for discrete glomeruli, homogeneous among 'sister' cells projecting to the same glomerulus and selective for particular odorants and sensitive to odorant concentrations. Using binary mixtures, we demonstrated that odorant-evoked inhibition can suppress the excitatory response to a different odorant and was present in populations of both mitral and tufted cells. Lastly, using mouse lines in which Channelrhodopsin-2 was targeted to olfactory sensory neurons expressing a single odorant receptor, we found that optical activation of inputs to a single glomerulus results in suppression of mitral cells in a small subset of surrounding glomeruli, demonstrating that interglomerular inhibition selectively targets some neighboring glomeruli while sparing others. These results

allow us to begin mapping inhibitory interactions across OB glomeruli and relating these interactions to odor coding space.

Disclosures: **M.N. Economo:** None. **K.R. Hansen:** None. **T. Bozza:** None. **M. Wachowiak:** None.

Nanosymposium

014. Plasticity in the Olfactory System

Location: 147A

Time: Saturday, November 15, 2014, 1:00 PM - 3:30 PM

Presentation Number: 14.08

Topic: D.01. Chemical Senses

Support: NIH Grant R01DC009948-01

NIH Grant RO1DC008701

Capes BEX 3214-13-3

Title: Effects of learning and neuromodulation in a computational model of olfactory bulb and cortex

Authors: ***C. LINSTER**, L. DE ALMEIDA;
CPL & Neurobio. and Behavior, Cornell Univ., Ithaca, NY

Abstract: Using a combined computational model of olfactory bulb and cortex, we investigate the effects of odor learning as well as cholinergic and noradrenergic modulation on odor processing and memory in olfactory cortex. We first show that olfactory bulb acetylcholine (ACh) modulates the sparseness and coherence of odor representations, leading to better readout and higher learning rates in olfactory cortex. ACh in olfactory cortex allows the formation of attractor memories by enhancing synaptic plasticity and neural excitability. Noradrenergic (NE) modulation in the OB modifies signal to noise ratio by decreasing spontaneous activity and enhancing odor responses. In olfactory cortex, NE modulates excitatory synaptic transmission as well as pyramidal cell and interneuron excitability and further enhances signal to noise ratio. To date, modeling results strongly predictive of behavioral results in the lab. Our model also shows how changes in PC neuronal parameters, experimentally shown to be induced by olfactory rule learning, modulate speed of acquisition and memory performance. We are currently exploring how regulation of ACh and NE occurs, how these two neuromodulators interact and how feedback projections from the PC to OB further modulate olfactory learning.

Disclosures: **C. Linster:** None. **L. de Almeida:** None.

Nanosymposium

014. Plasticity in the Olfactory System

Location: 147A

Time: Saturday, November 15, 2014, 1:00 PM - 3:30 PM

Presentation Number: 14.09

Topic: D.01. Chemical Senses

Support: NIDCD DC011184

Title: Mice use bilateral olfactory cues and adaptive sniffing to track odor trails

Authors: ***P. W. JONES**, N. N. URBAN;
Biol. Sci., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Spatial localization of odors is a behavior in which many animals engage, but one that has received relatively little attention in comparison to odor discrimination and detection tasks. Insects, dogs, rats, and even humans will follow surface-based scent trails, but this behavior hasn't previously been studied in mice. We demonstrate a behavioral task where the mouse must track an odor path in order to locate food rewards in a large arena. Video of the animal's movements is captured from below under infrared illumination of the clear arena surface. We automatically track the animal's body and nose position while also monitoring respiration via a thermocouple implanted in the mouse's nasal cavity. Animals quickly learn to continuously follow large sections of odor paths that are greater than 1 meter in length, and consistently engage odor paths associated with reward over unrewarded odor paths. Tracking fidelity plateaus after about 3 weeks of consistent training. We used reversible single nares occlusion to investigate if mice use bilateral olfactory cues to perform this task. We observe that tracking fidelity declines after occluding a single nares and that the animal's nose position while during tracking systematically shifts in the direction of the occluded nares (e.g. right side occlusions cause rightward positional shifts). During this task, the animals sniff at a high rate (>10 Hz) while exploring the trail. The observed sniff rate increases with increasing nose velocity, though this velocity-frequency relationship plateaus about halfway along the range of velocities observed at ~14-16 Hz sniff rates. Thus, mice adjust their sniff rate based on movement speed while engaged in olfactory tracking, within a range of speeds. Additionally, animals often follow the trail by sweeping their noses back and forth across its width, and we have been able to quantify this behavior and are investigating how precisely sniffing is correlated with these continuous searching movements. Though our respiration data seem consistent with sniffing

rates observed in rats during a similar task (Khan et al, 2012), they are quite different from the sniffing observed during most olfactory discrimination or detection paradigms, which are the conditions under which most single-cell electrophysiological recordings have been made to date. We plan obtain such recordings during this task, and efforts are underway to automate the reward allocation during the task in order to increase the amount of time the animal will spend on the task per day.

Disclosures: P.W. Jones: None. **N.N. Urban:** None.

Nanosymposium

014. Plasticity in the Olfactory System

Location: 147A

Time: Saturday, November 15, 2014, 1:00 PM - 3:30 PM

Presentation Number: 14.10

Topic: D.01. Chemical Senses

Title: Combining optogenetics and two photon calcium imaging to explore the functional impact of newborn neurons in the mouse olfactory bulb

Authors: *C. FOIS[1], G. PRAMANIK[1], S. PÉRON[2], B. BERNINGER[2], A. STROH[1];
[1]Focus Transl. Neurosc. (FTN) and Inst. for Microsc. Anat. and Neurobiol., [2]Adult Neurogenesis and Cell. Reprogramming, Johannes Gutenberg, Mainz, Germany

Abstract: The adult mammalian brain maintains the capability of generating new neurons within two neurogenic niches located in the subgranular zone (SGZ) of the hippocampus and in the subventricular zone (SVZ) of the lateral ventricles. Neuronal progenitors migrate from the SVZ via the rostral migratory stream (RMS) towards the olfactory bulb where they integrate into the pre-exisiting neuronal circuitry. The specific function of newborn neurons - mainly GABAergic interneurons - in the OB microcircuitry remains poorly understood. Here, we used 2-photon calcium imaging to monitor the optical correlate of neuronal spiking activity in a microcircuit of up to 100 neurons in the mouse OB *in vivo*, combined with specific optogenetic modulation of newborn neurons with the aim to address their influence on olfactory processing. We stereotactically injected Adeno-Associated Viruses (AAVs) encoding for the hyperpolarizing proton pump ArchT and the excitatory opsin ChR2 in the SVZ of adult mice. Within 40 days, neuronal stem cells expressing opsins migrated from the SVZ to the OB and differentiated into mature neurons, expressing neuronal markers such as NeuN. We found a strong membrane-bound expression of opsins in the newly integrated neurons in the OB, persisting for at least 7 months. We also injected the genetically encoded calcium indicator GCaMP6 in the external

plexiform and the mitral cell layer, representing the main output of the olfactory bulb circuitry. Using an open-window preparation, we can now image the spontaneous and odor-evoked activity with high temporal resolution of up to 40 Hz using a resonant scanner with optogenetic integration. With this tools at hand we will scrutinize the causal contribution of newborn neurons to olfactory processing in the adult olfactory bulb.

Disclosures: **C. Fois:** None. **G. Pramanik:** None. **S. Péron:** None. **B. Berninger:** None. **A. Stroh:** None.

Nanosymposium

015. Auditory Processing

Location: 206

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 15.01

Topic: D.02. Auditory

Support: NIH Grant R01NS044363

NIH Grant 1F31DC012013

NIH Grant R21DC006089

Title: A non-canonical pathway from cochlea to brain detects tissue-damaging noise and mediates auditory nociception

Authors: *J. GARCIA-ANOVEROS[1], E. N. FLORES[1], T. MADATHANY[1], G. KUMAR[1], M. C. LIBERMAN[2], R. SEAL[3], R. EDWARDS[3], A. DUGGAN[1];
[1]Anesthesiol, Physiol, Neurol, Northwestern Univ., CHICAGO, IL; [2]Otology and Laryngology, Harvard Med. School, Massachusetts Eye & Ear Infirmary, Boston, MA; [3]Neurol. and Physiol., UCSF, San Francisco, CA

Abstract: Sound causes vibration of the organ of Corti, the part of the cochlea where auditory inner and outer hair cells (IHCs and OHCs) reside. Stimulated IHCs release glutamate that activate type I afferents, the predominant primary sensory neurons of the cochlear spiral ganglion, and these in turn project to the cochlear nucleus. This pathway is how sound detected by the cochlea reaches the brain. In contrast, mechanically stimulated OHCs alter their shape and amplify the vibration of the organ of Corti, and hence augment stimulation of IHCs at low sound pressure levels, but they do not directly activate type I afferents. The only afferents under the OHCs anr type IIs, whose processes branch out and resemble that of somatosensory nociceptors,

but whose function is unknown. Although cochlear hair cells are specialized in detecting sound-induced mechanical stimulation, very loud and persistent noise will damage and even kill them. Throughout most of the body, somatosensory nociceptors of the dorsal and trigeminal ganglia detect tissue damage of this sort. However, somatosensory neurons do not innervate the organ of Corti, begging the questions of whether its damage goes undetected or whether the cochlea has alternative, nociceptor-like neurons that detect damage. To test these hypotheses, we used an animal model (Vglut3-/- mice) in which IHCs do not release glutamate and hence fail to activate type I afferents, completely disabling the canonical auditory pathway. We measured neuronal activity by cFos immunoreactivity in the cochlear nucleus (where cochlear afferents terminate) of Vglut3-/- deaf mice and found response to harmful (120 dB SPL at 8-16 kHz for 1 hr, which damages and kills hair cells), but not innocuous (80 dB SPL at 8-16 kHz for 1 hr), noise. This response originates in the cochlea and not in other areas also stimulated by loud noise (middle ear and vestibule) as it was absent in mice with selective cochlear degeneration (with nearly complete depletion on cochlear hair cells) but normal vestibular and somatosensory function. Hence, we found evidence for an alternative pathway between cochlea and brainstem that does not involve glutamate release from IHCs. This pathway responds to tissue-damaging noise, serving a novel form of sensation we term auditory nociception.

Disclosures: J. Garcia-Anoveros: None. **E.N. Flores:** None. **T. Madathany:** None. **G. Kumar:** None. **M.C. Liberman:** None. **R. Seal:** None. **R. Edwards:** None. **A. Duggan:** None.

Nanosymposium

015. Auditory Processing

Location: 206

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 15.02

Topic: D.02. Auditory

Support: NIH NIDCD R01DC011481

Title: Pulsed infrared radiation elicits transient changes in mitochondrial membrane potential in cultured neurons

Authors: V. LUMBRERAS[1], *S. RAJGURU[1,2];
[1]Dept. of Biomed. Engin., Univ. of Miami, Coral Gables, FL; [2]Otolaryngology, Univ. of Miami, Miami, FL

Abstract: Pulsed Infrared Radiation (IR) has been shown to elicit controllable intracellular calcium and electrical responses in neurons. This somatic excitability may be due to mitochondrial absorption of IR. Application of focused optical stimuli to target single cell activation and even subcellular mechanisms may lead to a broad range of basic science and prosthetic applications. It is therefore important to characterize how IR modulates mitochondrial properties in neurons. Towards this goal, we analyzed whether pulsed IR changes mitochondrial membrane potential, Ψm, activating mitochondrial Ca2+ cycling. Experiments were performed on cultured spiral and vestibular ganglion neurons isolated from p3 rat pups. The neurons were loaded and incubated for 30 minutes with Δψm sensors JC-1 (1.5 μM), Rhodamine 123 (10 μM, quenching), and TMRE (100 nM, nonquenching). Oligomycin A and FCCP were used as controls to confirm quenching and nonquenching behaviors. Pulsed IR was delivered to the neurons using a 400 μm optical fiber connected to a Capella laser (λ = 1863 nm). Both TMRE and Rhodamine 123 showed hyperpolarization of mitochondria under IR. CGP-37157, Ruthenium Red (50 μM, mitochondrial Ca2+ cycling blockers), and Bapta-AM (10 μM, Ca2+ chelator) inhibited pulse-by-pulse responses elicited by IR in Rhodamine 123. JC-1 fluorescence confirmed that the mitochondrial membrane potential had returned to resting level 24 hours after long-term stimulation with IR. Lack of cell damage was further confirmed by colocalization of mitochondria and cytochrome c at 6 hours and negative staining of cleaved-caspase 3 at 24 hours. Positive staining of ROS using CellROX reagent 6 hours after IR indicated mitochondrial energization. The results suggest that IR transiently hyperpolarizes and energizes mitochondria. Consequently, IR could be utilized to study the role of mitochondria on Ca2+ dynamics and synaptic transmission, respiratory metabolism, and mitochondrial signaling. Funding: NIH NIDCD R01DC011481.

Disclosures: **V. Lumbreras:** None. **S. Rajguru:** None.

Nanosymposium

015. Auditory Processing

Location: 206

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 15.03

Topic: D.02. Auditory

Support: NIH/R90 DA 033460

NIH/NIDCD R01 DC00100

Title: An auditory network model for spatial sound stream segregation

Authors: *J. DONG, K. SEN, H. S. COLBURN;
Biomed. Engin., Boston Univ., Boston, MA

Abstract: This modeling study aims to understand how the brain segregates spatial sound sources - a key step in solving the cocktail party problem. Although the benefits of spatial cues in separating target stimuli from interfering maskers have been demonstrated in behavioral studies, the neural mechanisms are not yet fully understood. We present a physiologically based computational network model to explain how auditory cortical neurons develop spatial tuning to, and segregate, spatially separated target and masker sources. The model is based on a previous study in the zebra finch Field L, which showed that the presentation of a competing masker increased spatial sensitivity to target sounds in Field L cortical neurons (Maddox et al., 2012, PLoS biology). The model is composed of spatial channels corresponding to the locations of target and masker stimuli, and uses lateral inhibition to suppress sources across spatial channels. We demonstrate that the model can generally explain the spatial responses of the majority of the recorded neurons. Based on the model, we propose testable predictions for explicit physiological experiments. In addition, we extend the model to construct an engineering solution that may be useful for hearing assistive devices for dealing with everyday mixed source environments. We use the network to segregate mixed-source speech waveforms, using stimulus reconstruction methods to convert the model output back to speech, and present the segregated speech to listeners.

Disclosures: J. Dong: None. **K. Sen:** None. **H.S. Colburn:** None.

Nanosymposium

015. Auditory Processing

Location: 206

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 15.04

Topic: D.02. Auditory

Support: Wellcome Trust Principal Research Fellowship

Newton Abraham Studentship

Title: Complementary mechanisms contribute to the developmental plasticity of spatial hearing

Authors: *P. E. KEATING, J. C. DAHMEN, A. J. KING;
Univ. Oxford, Oxford, United Kingdom

Abstract: Spatial hearing evolved independently in mammals and birds, and is thought to adapt to altered developmental input in different ways. Whereas barn owls adapt to a unilateral hearing loss through a compensatory adjustment in neuronal sensitivity to abnormal binaural spatial cues, mammals appear to ignore these cues and instead become more dependent on the unchanged spectral cues available at the intact ear. It is unclear, however, whether mammals can adapt to abnormal binaural cues if spectral cues are unavailable. We therefore reared ferrets with an earplug in one ear and measured the ability of these animals to localize high-frequency narrowband sounds that limited the availability of spectral cues. When wearing an earplug in the developmentally-occluded ear, they made smaller errors than acutely-plugged controls. Although errors were larger than controls with normal hearing, this indicates that ferrets partially adapted to abnormal binaural cues at high frequencies. To determine whether neurophysiological changes in interaural level difference (ILD) sensitivity might underlie this ability, we measured the ILD sensitivity of units in the primary auditory cortex (A1) of control and juvenile-plugged ferrets in the presence or absence of a virtual earplug in the developmentally-occluded ear. Relative to controls, units recorded in juvenile-plugged ferrets showed greater ILD sensitivity, with both left and right hemispheres exhibiting a preference for ILDs contralateral to the occluded ear. This implies that ILDs cannot be represented by simply comparing activity in the two hemispheres. Instead, intra-hemispheric comparisons between units with different ILD preferences are likely to be crucial for maintaining ILD sensitivity, which we confirmed by analyses of neuronal activity at a population level. Since ferret A1 is already known to adaptively reweight auditory spatial cues following a developmental hearing loss in one ear, our results suggest that developmental plasticity may be driven by adjusting sensitivity to ILDs, while simultaneously down-weighting this binaural cue when intact spectral cues are available. These two mechanisms are likely to complement one another by enabling robust localization of diverse sound types in different acoustic environments.

Disclosures: **P.E. Keating:** None. **A.J. King:** None. **J.C. Dahmen:** None.

Nanosymposium

015. Auditory Processing

Location: 206

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 15.05

Topic: D.02. Auditory

Support: NSF Grant 333-1131

Title: Neural mechanisms for preserving information about multiple sound items

Authors: *V. C. CARUSO[1,2], J. LEE[6], D. S. PAGES[2], R. ESTRADA[3], J. M. GROH[1,2,4], S. TOKDAR[5];
[1]Ctr. For Cognitive Neurosci., [2]Psychology and Neurosci., [3]Dept. of Ophthalmology, [4]Neurobio., [5]Dept. of Statistical Sci., Duke Univ., Durham, NC; [6]Psychology, Univ. of Canterbury, Christchurch, New Zealand

Abstract: We often perceive more than one item at a time, but how we do so is poorly understood, particularly when the stimuli involved recruit overlapping populations of neurons. One way to encode multiple simultaneous items is through multiplexing, in time or across neural assemblies. Time multiplexing would entail interleaved responses in a single signal. That is, individual neurons might switch between representing each of multiple items at some unknown time scale. Neural assembly multiplexing would entail each individual neuron encoding only one item, with each item encoded by a different group of neurons. Either or both mechanisms could allow a neural population to preserve information about multiple items of information. We address this issue using sound localization as a model system. The location (azimuth) of single sounds is encoded in the primate brain via firing rates that are proportional to and thus can encode sound azimuth (i.e. a "meter" as opposed to a map for sound location). This poses a capacity problem for multiple simultaneous locations. We focus on the Inferior Colliculus (IC), an essential node in the auditory pathway. Any limitations in the coding capacity of the IC are likely to be inherited by subsequent auditory areas. Two monkeys were trained to report the location of two simultaneous sounds at different frequencies, confirming that the two locations are successfully perceived and thus must somehow be encoded (Lee et al. SfN 2013). During the localization task, the spiking activity of single cells and local field potentials were recorded in the IC. We asked whether IC neurons multiplex representations of different sounds in their spike trains - within (time multiplexing) or between trials (neural assembly multiplexing). A Hidden Markov Model analysis (HMM), which makes no assumption on the time scale or repeatability of switching across trials, supports time multiplexing within trials for 23% of the tested cells. In addition, an aggregate-count analysis (Lee et al 2013) provides evidence that a different sub-population (41% cells) switches across trials (neural assembly multiplexing). In total, evidence for multiplexing was seen in ~ 65% of the tested cells. We next asked if neural oscillations might be related to the mechanism that accomplishes this switching within and/or across trials. A correlation analysis between spike trains of individual cells and oscillatory signals in the LFPs revealed that early responses to sounds varied with the phase of the LFP in the 25-40Hz band. Together, these results support the multiplexing hypothesis and implicate neural oscillations as a signature related to the underlying mechanism.

Disclosures: V.C. Caruso: None. **J. Lee:** None. **D.S. Pages:** None. **R. Estrada:** None. **J.M. Groh:** None. **S. Tokdar:** None.

Nanosymposium

015. Auditory Processing

Location: 206

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 15.06

Topic: D.02. Auditory

Support: NIDCD Grant 5R01DC04199

Title: Development of intrinsic connectivity in the central nucleus of the mouse inferior colliculus

Authors: *J. J. STURM[1], T. NGUYEN[2], K. KANDLER[1];
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Abstract: The central nucleus of the inferior colliculus (CNIC) is a tonotopically-organized midbrain nucleus that serves as a major subcortical site of integration for auditory processing. The CNIC contains a dense and complex network of local, intra-collicular connections, which are thought to both provide gain control and contribute to the emergence of selectivity for complex acoustic features. However, very little is known about the functional organization of intrinsic connections in the CNIC and their development. As a first step towards elucidating the functional organization of these local networks, we characterized the strength and the spatial distribution of local excitatory and inhibitory inputs received by CNIC neurons during the first two weeks of postnatal development (postnatal days 2 - P15). Using scanning laser photostimulation with caged glutamate we demonstrate the presence of extensive excitatory and inhibitory intra-collicular connectivity already at P2. Both excitatory and inhibitory synaptic inputs formed continuous maps that largely overlapped with each other and that were aligned with the presumed isofrequency axis of the CNIC. Although this characteristic organization was present throughout the first two postnatal weeks, the size of input maps was developmentally regulated as input maps underwent an expansion during the first week that was followed by a dramatic refinement around hearing onset. These changes occurred in parallel for both excitatory and inhibitory input maps. However, the functional elimination of intrinsic connections was greater for excitatory than for inhibitory connections, resulting in a predominance of intrinsic inhibition at hearing onset.

Disclosures: J.J. Sturm: None. **T. Nguyen:** None. **K. Kandler:** None.

Nanosymposium

015. Auditory Processing

Location: 206

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 15.07

Topic: D.02. Auditory

Support: NIH; R01DC008983

David and Lucile Packard Foundation

NIH grant R01EY019049

Title: Scaling down of balanced excitation and inhibition by active behavioral states in auditory cortex

Authors: *M. ZHOU[1], F. LIANG[1,2], X. XIONG[1], L. LI[2], H. LI[2], Z. XIAO[2], H. TAO[1], L. ZHANG[1];
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Abstract: Cortical sensory processing is modulated by behavioral and cognitive states. How this modulation is achieved by changing synaptic circuits remains largely unknown. In awake mouse auditory cortex, we found that sensory-evoked spike responses of layer 2/3 (L2/3) excitatory cells were scaled down with preserved sensory tuning when mice transitioned from quiescence to active behaviors, including locomotion, whereas L4 and thalamic responses were unchanged. Whole-cell voltage-clamp recordings revealed that tone-evoked synaptic excitation and inhibition exhibited a robust functional balance. The change to active states caused scaling down of excitation and inhibition at approximately equal levels in L2/3 cells, but resulted in no synaptic changes in L4 cells. This lamina-specific gain control could be attributed to an enhancement of L1-mediated inhibitory tone, with L2/3 parvalbumin inhibitory neurons also being suppressed. Thus, L2/3 circuits can adjust the salience of output in accordance with momentary behavioral demands while maintaining the sensitivity and quality of sensory processing.

Disclosures: **M. Zhou:** None. **F. Liang:** None. **X. Xiong:** None. **L. Li:** None. **H. Li:** None. **Z. Xiao:** None. **H. Tao:** None. **L. Zhang:** None.

Nanosymposium

015. Auditory Processing

Location: 206

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 15.08

Topic: D.02. Auditory

Support: DC011284 to DHS and VCK

DC013482 to TMM

Title: A critical period for auditory recipient striatum differs from primary auditory cortex

Authors: *T. M. MOWERY, V. C. KOTAK, D. H. SANES;
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Abstract: Background The central nervous system displays many brief epochs of increased plasticity, known as critical periods (CP), and these have been studied almost exclusively in the primary sensory cortices. We identified the region of striatum that receives input from the primary auditory cortex, using an anterograde tracer (Fluoro Ruby), and generated a brain slice that contained the thalamo-cortico-striatal pathway. Our previous studies have demonstrated that mild (~25 dB) developmental hearing loss induces significant changes to cortical inhibitory postsynaptic currents (IPSCs), and the window of sensitivity opens by P11 and is closed at P18 (Mowery et al., 2013 - SFN). Therefore, we were interested in how such transient hearing loss affects IPSCs in a region of the striatum that receives cortical input from the primary auditory cortex. Methods Whole-cell voltage clamp recordings of spontaneous IPSCs were obtained from auditory recipient putative medium spiny neurons in thalamo-cortico-striatal slice preparations from gerbils (Meriones unguiculatus) that underwent mild hearing loss. Animals had earplugs (EP) inserted bilaterally on postnatal day (P)11, P17, or P18. Recordings were obtained from EP and control brain slices at P29 to 35. Results When ears were plugged from P11 to the day of recording, spontaneous IPSC amplitude and decay time constant were not significantly affected (sIPSC amp: Ctrl= -23.3 ± 3.3 vs. EP11= -20.1 ± 1.4 mV, $p>0.05$; sIPSC tau decay: Ctrl= 21.1 ± 1.5 vs. EP11= 23.5 ± 1.9 ms, $p>0.05$). However, the sIPSC frequency was significantly diminished in animals reared with EPs (sIPSC freq: Ctrl= 10.4 ± 1.8 vs. EP11= 2.05 ± 0.6 Hz, $p<0.001$). Furthermore, the window of plasticity remained open after the CP of plasticity has closed in the cortex, i.e. beyond P18. Thus, sIPSC frequency was significantly reduced when EPs were inserted at P18, (Ctrl= 10.4 ± 1.8 vs. EP18= 4.7 ± 0.8 Hz, $p<0.05$). Conclusion These results suggest that mild hearing loss affects inhibitory synapse development in auditory recipient striatum. Specifically, the profound decrease in the rate of sIPSCs strongly implies that

release probability of GABA in medium spiny neurons may be influenced by auditory experience. Furthermore, the fact that the critical period window within the striatum remained open past the closure of the cortical CP suggests that critical period plasticity may differ between interconnected regions of a sensory neuraxis.

Disclosures: T.M. Mowery: None. **V.C. Kotak:** None. **D.H. Sanes:** None.

Nanosymposium

015. Auditory Processing

Location: 206

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 15.09

Topic: D.02. Auditory

Support: 2013 NARSAD Young Investigator Grant

NIH NIDCD R03 DC 013660

Burroughs Wellcome Career at the Scientific Interface Award

Klingenstein Fellowship in Neuroscience

Pennsylvania Lions Club Hearing Research Fellowship

Title: Cortical inhibition regulates frequency discrimination acuity and specialization of emotional learning

Authors: *M. AIZENBERG[1], L. MWILAMBWE-TSHILOBO[2], M. GEFFEN[2];
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Abstract: Hearing perception relies on our ability to tell apart the spectral content of different sounds, and to distinguish behaviorally relevant sounds. A number of recent studies, conducted in both human patients and animal models, suggest an important role for AC in frequency discrimination and emotional auditory learning. Recently, we found that the auditory cortex regulates frequency discrimination acuity following discriminative fear conditioning (Aizenberg and Geffen, 2013). However, the neuronal circuits that underlie this modulation remain unknown. In the auditory cortex, excitatory neurons serve the dominant function in transmitting information about the sensory world within and across brain areas, whereas inhibitory interneurons carry a range of modulatory functions, shaping the way information is represented

and processed. Here, we demonstrate that the most common class of interneurons, parvalbumin-positive (PVs), modulate frequency selectivity of excitatory neurons in AC, and regulate behavioral frequency discrimination acuity and specificity of emotional learning. To measure the effect of PV activity, we activated or inhibited PVs selectively and temporally precisely by shining light on the cortex of mice that were driven to express Channel- or Archae-rhodopsin in PC neurons through a Cre-dependent viral delivery system. At the same time, we either recorded neuronal activity in AC in awake subjects, or performed behavioral testing. Frequency selectivity of neurons increased following activation of PVs and decreased following inhibition of PVs. Behavioral frequency discrimination threshold was determined using a modified procedure for measurement of the pre-pulse inhibition of the startle reflex. Moderate activation of PVs decreased frequency discrimination threshold, while inhibition of PVs increased the threshold. Modulating PV activity also affected the specificity of discriminative auditory fear conditioning: Inhibiting PVs during conditioning led to a decrease in specificity of the fear responses to the frequency of the conditioned tone. The effect of PVs on auditory behavior was consistent with the measured changes in frequency tuning of AC neurons. These results significantly expand our understanding of how specific cortical circuits contribute to auditory perception and emotional learning. Understanding the function of these circuits in normal hearing will help identify potential cell-level targets for treatments of hearing and communication, as well as emotional disorders. Reference M. Aizenberg and M. N. Geffen (2013) Nat. Neurosci. 16, 994-996

Disclosures: **M. Aizenberg:** None. **L. Mwilambwe-Tshilobo:** None. **M. Geffen:** None.

Nanosymposium

015. Auditory Processing

Location: 206

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 15.10

Topic: D.02. Auditory

Title: Unconsciously implanted memory in the presence of cholecystokinin retrieved in a behaviorally relevant context

Authors: ***Z. ZHANG**[1], D. LU[3], X. LI[3], X. CHEN[3], W. SUN[3], Y. GUO[4], G. NG[2], J. HE[3];
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Abstract: In a previous study, we established a long-term visuoauditory associative memory in rats after pairing a visual stimulus with electrical stimulation of the auditory cortex. In the present study we investigated whether such associative memory can be artificially implanted under anesthesia and then retrieved in a behaviorally relevant context. Rats with bilateral electrodes implanted in the auditory cortex were trained to approach the left or right hole of a behavioral apparatus to retrieve a reward depending on whether the right or left auditory cortex was electrically stimulated. An initially irrelevant light stimulus was then repeatedly paired with electrical stimulation of the auditory cortex in one hemisphere after infusion of cholecystokinin (CCK) under anesthesia, potentially establishing an artificial link between the light stimulus and the stimulation of the CCK-infused hemisphere. After intervention, auditory cortex neurons of this hemisphere started to respond to the light stimulus in both anesthetized and awake states. In subsequent behavioral testing, in response to the light stimulus, rats approached the hole that was "engineered" to be associated with reward availability. Three control experiments showed that this behavioral change was not caused by non-specific effects of CCK infusion or pairings of stimuli alone. Moreover, subsequent stimulus pairings in the contralateral hemisphere resulted in a switch in the direction of behavior, demonstrating the flexibility of the auditory cortex. Our finding that an artificial associative memory formed in the neocortex under anesthesia could be translated into behavioral action provides a scientific foundation for "memory implantation".

Disclosures: Z. Zhang: None. **D. Lu:** None. **X. Li:** None. **X. Chen:** None. **W. Sun:** None. **Y. Guo:** None. **G. Ng:** None. **J. He:** None.

Nanosymposium

015. Auditory Processing

Location: 206

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 15.11

Topic: D.02. Auditory

Support: Natural Sciences and Engineering Research Council of Canada

Tinnitus Research Initiative

Title: Expression of neural plasticity and attention in normal hearing is modified in tinnitus

Authors: *L. E. ROBERTS[1], B. T. PAUL[2], I. C. BRUCE[3], D. J. BOSNYAK[2];
[2]Psychology Neurosci. and Behaviour, [3]Electrical and Computer Engin., [1]McMaster Univ., Hamilton, ON, Canada

Abstract: Most if not all models of tinnitus generation propose that neural plasticity contributes to the neural changes that underlie tinnitus. It has also been proposed that the disparity between what the auditory cortex predicts it should be hearing (this prediction coded by aberrant synchronous neural activity occurring in primary auditory cortex and coding for the tinnitus percept) and input delivered to the brain by the damaged cochlea activates neural systems that support auditory attention (Roberts, Husain, and Eggermont, Neuroscience and Biobehavioural Reviews, 37:1754-1773). Our research has investigated how the expression of neural plasticity and attention in the normal hearing human brain (derived from laboratory training experiments and studies of musicians using high resolution EEG) appears to be modified in individuals experiencing tinnitus. The findings support the view that the rules that describe auditory remodeling in the normal hearing brain are modified by the presence of tinnitus-related neural activity occurring in auditory pathways. Tinnitus-related modifications include a relaxation of constraints on auditory representations in the tinnitus frequency region of primary auditory cortex, impaired temporal plasticity in subcortical pathways, and reduced modulation by attention of brain responses in the tinnitus frequency region of primary auditory cortex and in nontonotopic secondary auditory cortex.

Disclosures: **L.E. Roberts:** None. **B.T. Paul:** None. **I.C. Bruce:** None. **D.J. Bosnyak:** None.

Nanosymposium

015. Auditory Processing

Location: 206

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 15.12

Topic: D.02. Auditory

Support: Spanish BFU2013-43608-P

Lejeune Foundation

Title: Stimulus-specific adaptation and novelty detection in a Down syndrome mouse model overexpressing Dyrk1A

Authors: ***D. DUQUE**[1], M. MARTÍNEZ DE LAGRÁN[2], M. DIERSSEN[2], M. MALMIERCA[3]; [1]Inst. De Neurociencias De Castilla Y León, Uni, Salamanca, Spain; [2]Ctr. for Genomic Regulation (CRG), Univ. Pompeu Fabra, Ctr. de Investigación Biomédica en Red de Enfermedades Raras, Barcelona, Spain; [3]Inst. De Neurociencias De Castilla Y León, Univ. de Salamanca, Salamanca, Spain

Abstract: Down syndrome is a genetic disease characterized by the presence of an extra copy of the human chromosome 21. Several mice models have been developed over the last years to understand this disease. The overexpression of the dual-specificity tyrosine (Y)-regulated kinase (Dyrk1A) in transgenic mice (TgDyrk1A), is sufficient to recapitulate Down syndrome-like features including motor abnormalities and cognitive deficits. Interestingly Down syndrome and autism spectrum disorders share some cognitive deficiencies affecting brain regions involved in learning and memory and Dyrk1A is a relevant genetic locus for both. The mismatch negativity (MMN) is a late-latency component of the auditory evoked related potentials elicited by an oddball paradigm that signals auditory change detection and other forms of novelty detection. Among other deficits, human subjects suffering of autism spectrum disorders show MMN abnormalities. Stimulus-specific adaptation (SSA) is a phenomenon similar to MMN but elicited at the cellular level, and therefore, has been suggested to be part of the neuronal mechanisms necessary to MMN formation. SSA occurs from the auditory midbrain (inferior colliculus) up to the cortex. We speculate that, besides MMN abnormalities, SSA dysfunctions may be present in developmental disorders such as Down syndrome and autism spectrum disorders. Currently, it remains unexplored if transgenic mice overexpressing Dyrk1A exhibit autistic subphenotypes, e.g., auditory change detection abnormalities. Therefore, here we aimed to test if TgDyrk1A and wild type (C57BL6/SJL) mice show differences in SSA coding. We recorded single unit activity in the inferior colliculus of the wild type and Dyrk1A mice to an oddball paradigm similar to that used in the MMN studies to characterize and compare SSA in both genotypes, using an awake preparation. Our preliminary data show lower levels of SSA in the transgenic mice overexpressing Dyrk1A compared with the wild type. These abnormalities demonstrate that some of neurological alterations that lead to cognitive deficiencies in the Down syndrome may be due to an abnormal coding of neuronal adaptation.

Disclosures: D. Duque: None. **M. Martínez de Lagrán:** None. **M. Dierssen:** None. **M. Malmierca:** None.

Nanosymposium

11. Autism Synaptic and Cellular Mechanisms

Location: 144A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 11.01

Topic: C.06. Developmental Disorders

Support: HHMI grant 55007654

Title: Antagonism of metabotropic glutamate receptors reverses autistic behaviours caused by exacerbated mRNA translation initiation

Authors: *A. AGUILAR VALLES[1,2], E. MATTA CAMACHO[2], G. LING[2], K. NADER[2], J.-C. LACAILLE[1], N. SONENBERG[2];
[1]Univ. De Montreal, Montreal, QC, Canada; [2]McGill Univ., Montreal, QC, Canada

Abstract: Exacerbated mRNA translation during brain development is associated to several forms of autism spectrum disorders (ASD). We recently demonstrated that deletion of the eukaryotic Initiation Factor 4E-binding protein 2 (4E-BP2), a negative regulator of mRNA translation initiation, leads to an imbalance in excitatory-to-inhibitory neurotransmission and ASD-like behaviours. Antagonism of type I metabotropic glutamate receptors (mGluR, including mGluR1 and 5) has been successfully used to reverse ASD phenotypes in mouse models of Fragile X syndrome and in clinical trials. Importantly, these receptors activate mRNA translation initiation and elongation. We investigated the potential of treating autistic-like phenotypes by antagonists of mGluR1 (JNJ 16259685) and mGluR5 (Fenobam) in 4E-BP2 null mice. A single dose of mGluR1 (0.3 mg/kg) or mGluR5 (3 mg/kg) antagonists, which we established inconsequential in wild type mice, was sufficient to reverse the deficits in social exploration and exacerbated repetitive behaviours (marble burying and self-grooming) in 4E-BP2 knock outs. Exacerbated hippocampal long term depression (LTD), a form of synaptic plasticity that is translation dependent, was also normalized by either antagonist. Interestingly, although LTD becomes protein synthesis independent in 4E-BP2 mice (i.e. insensitive to elongation blocker anisomycin) this same treatment restored the levels of LTD back to those of wild type, suggesting that translational regulation downstream of initiation maybe amenable for regulation in 4E-BP2 null mice and can be a target of type I mGluR antagonists. We demonstrated that antagonism of type I mGluRs is a potential therapy that extends to autism models involving exacerbated mRNA translation initiation.

Disclosures: **A. Aguilar Valles:** None. **E. Matta Camacho:** None. **G. Ling:** None. **K. Nader:** None. **J. Lacaille:** None. **N. Sonenberg:** None.

Nanosymposium

11. Autism Synaptic and Cellular Mechanisms

Location: 144A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 11.02

Topic: C.06. Developmental Disorders

Support: SFARI 206683

Title: Repetitive behaviors in mice with specific deletion of Grip1/2 in Purkinje cells

Authors: *R. M. MEJIAS-ESTEVEZ[1], S.-L. CHIU[2], R. ROSE[3], M. HAN[3], R. L. HUGANIR[2], T. WANG[4];
[2]Neurosci., [3]Inst. of Genet. Med., [4]Pediatrics, [1]Johns Hopkins Univ., Baltimore, MD

Abstract: Cerebellar Purkinje cell (PC) loss and dysfunctions have been implicated in autism pathogenesis, although the molecular mechanisms are poorly understood. Glutamate receptor interacting protein 1 and 2 (GRIP1/2) are scaffolding proteins that regulate AMPA receptors recycling and synaptic transmission in the cerebellum. Previous studies from our laboratory have identified autism-associated mutations at Grip1/2 that result in more severe phenotype in autism patients. To study the role of GRIP1/2-mediated glutamate signaling at PCs in autism-associated phenotype, we have produced Purkinje cell-specific Grip1/2 double knockout (DKO) mice by crossing Grip2 conventional knockout (KO) and Grip1 conditional KO with Let-7 Cre mice. A pilot study showed that these DKO mice appeared to have normal number and morphology of Purkinje cells in cerebellum. We carried out a battery of behavior tests to study the phenotype of PC-specific Grip1/2 DKO male mice. Mutant mice showed normal levels of ambulatory activity, anxiety, sociability, and social novelty as compared to their wt littermates. Intriguingly, PC-specific Grip1/2 DKO mice presented a significant increase in cumulative time of stereotypic and repetitive grooming as compared to wt mice, and a mild deficit in motor and/or balance but not in motor learning in rotarod test. Increased stereotypic grooming in mice is a well-established, autism-specific behavior in autism mouse model. Our studies on PC-specific Grip1/2 KO mice suggest a crucial role of Grip1/2-mediated AMPA glutamate-signaling at PCs in stereotypic grooming behaviors in mice. Results from this study will provide valuable insights into the pathogenesis of stereotypic behaviors associated with autism.

Disclosures: R.M. Mejias-Estevez: None. **S. Chiu:** None. **R. Rose:** None. **M. Han:** None. **R.L. Huganir:** None. **T. Wang:** None.

Nanosymposium

11. Autism Synaptic and Cellular Mechanisms

Location: 144A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 11.03

Topic: C.06. Developmental Disorders

Title: Phenotypic effects of MeCP2 deletion in cholinergic neurons

Authors: *E. BALLINGER[1], C. SCHAAF[2], D. TALMAGE[3], H. Y. ZOGHBI[2,4,5], L. ROLE[3,6,7]; [1]Grad. Program in Neurosci., Stony Brook Neurosci., Stony Brook, NY; [2]Mol. and Human Genet., Baylor Col. of Med., Houston, TX; [3]Neurobio. and Behavior, Stony Brook Univ., Stony Brook, NY; [4]Howard Hughes Med. Inst., Chevy Chase, MD; [5]Jan and Dan Duncan Neurolog. Res. Inst. at Texas Children's Hosp., Houston, TX; [6]Ctr. for Nervous Syst. Disorders, Stony Brook, NY; [7]Neurosciences Inst., Stony Brook, NY

Abstract: Rett Syndrome (RTT) is an autism spectrum disorder that affects approximately 1 in 20,000 girls and is caused by mutations in the gene encoding methyl CpG binding protein 2 (*MeCP2*). The cholinergic system appears to be particularly important in RTT, as decreases in cholinergic markers have been correlated with increased clinical severity in patients with RTT. Schaaf and Zoghbi have developed a powerful transgenic mouse model, whereby *MeCP2* is selectively deleted in cholinergic neurons only, to facilitate study of the contribution of this cholinergic lesion to the overall phenotype of RTT. Interestingly, this model exhibits a selective deficit in recognition memory, a form of declarative memory that has been shown by lesion and electrophysiological studies to be dependent upon cholinergic signaling in the perirhinal cortex (PRH). This memory deficit may map onto the intellectual disability seen in patients with RTT, however, its molecular and electrophysiological underpinnings are unknown. We use optogenetics and in vivo electrophysiology to selectively activate cholinergic neurons in the Nucleus Basalis of Meynert (NBM), the cholinergic source nucleus that innervates the PRH, while simultaneously recording the effects of this selective activation in the PRH. We have demonstrated not only that NBM opto-stimulation modulates both the rate and variability of firing among PRH neurons, but that this modulation is impaired among selective cholinergic *MeCP2* knock-out mice. Additionally, we have found a decrease in expression of ChAT, the cholinergic synthetic enzyme, among selective *MeCP2* knock-out mice specifically in the NBM, while expression in other cholinergic nuclei is spared. This suggests that the recognition memory deficit seen among cholinergic *MeCP2* knock-out mice is mediated by deficient acetyl choline synthesis and signaling in the NBM-PRH circuit and that other cholinergic nuclei are robust to *MeCP2* deletion. These results may help guide the development of future targeted treatment strategies for patients with RTT.

Disclosures: E. Ballinger: None. **C. Schaaf:** None. **H.Y. Zoghbi:** None. **D. Talmage:** None. **L. Role:** None.

Nanosymposium

11. Autism Synaptic and Cellular Mechanisms

Location: 144A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 11.04

Topic: C.06. Developmental Disorders

Support: NIH Grant R01 DA017392

IDDRC Pilot Grant-NIH P30 HD071593

Title: Dysregulation of hippocampal inhibition in the CNTNAP2 knockout mouse

Authors: *S. JURGENSEN[1], P. E. CASTILLO[2];
[1]Dominick P. Purpura Neurosci. Dept., [2]Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., New York, NY

Abstract: Mutations in the gene encoding contactin-associated protein-like 2 (*CNTNAP2*) have been strongly associated with Autism Spectrum Disorders (ASDs). In particular, individuals carrying recessive *CNTNAP2* mutations tend to display language impairment and epilepsy phenotypes. *CNTNAP2*$^{-/-}$ mice represent a good model to study ASDs, as they reproduce the three core behavioral endophenotypes: social impairment, language deficits and repetitive behavior (Peñagarikano et al. 2011, Cell). In addition, *CNTNAP2*$^{-/-}$ mice show signs of asynchronous neuronal activity in the cortex and develop spontaneous seizures throughout adulthood, raising the possibility of an existing imbalance in synaptic activity. Despite its widespread expression in the brain, the only known function of the protein encoded by *CNTNAP2*, CASPR2, is to cluster potassium channels in the juxtaparanodes of myelinated axons in the PNS (Poliak et al., 1999, Neuron). Knockdown of *CNTNAP2* in cultured hippocampal neurons results in generalized decreases in multiple parameters of both excitatory and inhibitory synaptic transmission (Anderson et al., 2012, PNAS). However, the consequence of lifelong deletion of *CNTNAP2* to synaptic function in the intact brain remains unknown. In the present study, we have assessed basic synaptic transmission in acute slices of the hippocampus of *CNTNAP2*$^{-/-}$ mice. Strikingly, when compared to their wild-type littermates, *CNTNAP2*$^{-/-}$ mice show normal excitatory synaptic transmission at the Schaffer collateral to CA1 pyramidal neuron synapse, as assessed by normal input-output function, paired-pulse ratio, burst-mediated synaptic depression, NMDAR-AMPAR ratio, and mEPSC activity. In contrast, inhibition onto CA1 pyramidal cells was abnormal in *CNTNAP2*$^{-/-}$ mice as indicated by a rightward shift in the input-output function of evoked IPSCs, and an increase in the frequency, but not amplitude of spontaneous IPSCs. Remarkably, neither the frequency nor the amplitude of mIPSCs were affected in *CNTNAP2*$^{-/-}$ mice, suggesting that the increase in spontaneous IPSCS could be due to hyperactive interneurons, a possibility that we are currently investigating. Changes in hippocampal inhibition could account for the epileptic phenotype developed by *CNTNAP2*$^{-/-}$ mice later in life, and potentially reflect a role for CASPR2 in clustering K^+ channels in interneurons. Overall, our findings suggest that *CNTNAP2* deletion affects hippocampal

inhibition but not excitation. These findings provide further insights into the precise alterations in synaptic connectivity observed in ASDs, and could ultimately help elucidate the cellular and synaptic basis underlying these disorders.

Disclosures: S. Jurgensen: None. **P.E. Castillo:** None.

Nanosymposium

11. Autism Synaptic and Cellular Mechanisms

Location: 144A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 11.05

Topic: C.06. Developmental Disorders

Support: KO8 NINDS

Hearst Foundation Fellowship

Title: Identification of critical periods for treatment of autistic behavior in purkinje cell Tsc1 mice

Authors: *P. TSAI[1], Y.-X. CHU[2], B. BLUDEVICH[1], J. MOGAVERO[1], W. REGEHR[2], M. SAHIN[1];
[1]Neurol., Boston Children's Hosp., Boston, MA; [2]Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Background: Cerebellar Dysfunction has been implicated in the pathogenesis of autism spectrum disorders. By generating a mouse model where Tsc1 is deleted specifically in cerebellar Purkinje Cells, we have recently demonstrated that cerebellar dysfunction is sufficient to generate abnormal autistic behaviors and that early treatment with the mTOR inhibitor rapamycin can prevent the development of cerebellar pathology and autistic-like behavior. Objectives: Evaluate the benefits of later rapamycin treatment on autistic-like behaviors and to delineate critical periods of treatment for autistic-like behaviors. Methods: Using our Purkinje Cell Tsc1 mouse mutants, we have investigated the impact of rapamycin treatment initiated during adulthood on behavior, pathology, and electrophysiologic function to delineate the critical periods of treatment of autistic behavior. Results: With rapamycin treatment starting at 6 weeks, we have demonstrated rescue of motor learning deficits and social behaviors - but not repetitive behaviors or cognitive inflexibility - in Purkinje Cell Tsc1 mutant mice. Rapamycin treatment at this time point also rescues pathologic and electrophysiologic deficits in these mice. Conclusion: These findings demonstrate that later treatment - even into adulthood - might offer benefit for

social impairments. Furthermore, we demonstrate a critical period for treatment of social behaviors that differs from the critical period of rescue for motor learning, repetitive behaviors, and cognitive inflexibility, providing a platform for investigating the mechanisms underlying the critical periods for these behaviors and for further investigating the cerebellar contribution to autistic behavior.

Disclosures: **P. Tsai:** None. **Y. Chu:** None. **B. Bludevich:** None. **J. Mogavero:** None. **W. Regehr:** None. **M. Sahin:** 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.; Novartis Inc..

Nanosymposium

11. Autism Synaptic and Cellular Mechanisms

Location: 144A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 11.06

Topic: C.06. Developmental Disorders

Support: JSPS, Postdoctoral Fellowships for Research Abroad

International Rett Syndrome Foundation

NIH/NINDS 5R01NS057819-08

Howard Hughes Medical Institute

Title: Dissecting the roles of parvalbumin and somatostatin-positive interneurons in the pathogenesis of Rett syndrome

Authors: *A. ITO-ISHIDA[1,2], K. URE[1,2], H. Y. ZOGHBI[1,2,3];
[1]Mol. and Human Genet., Baylor Col. of Med., Houston, TX; [2]Jan and Dan Duncan Neurolog. Res. Inst. at Texas Children's Hosp., Houston, TX; [3]Howard Hughes Med. Inst., Houston, TX

Abstract: Rett syndrome (RTT) is an X-linked neurodevelopmental disorder caused by mutations of the gene encoding methyl-CpG-binding protein 2 (MeCP2). Typical symptoms of RTT are normal early development followed by regression, repetitive hand wringing, motor dysfunction, autistic features, and autonomic dysfunction. To understand the pathogenesis of RTT, our group has characterized multiple mouse lines that lack MeCP2 in specific subsets of neurons. Using this approach, it was discovered that mice lacking MeCP2 in inhibitory neurons

recapitulate the majority of RTT symptoms (Chao et al., 2010, Nature). In addition, we have recently found that expressing MeCP2 selectively in inhibitory neurons was sufficient to rescue many RTT-related phenotypes (unpublished data). These results indicate that MeCP2 function in inhibitory neurons is critical for normal brain function and that its loss in this neuronal population is a key to RTT pathogenesis. Inhibitory neurons are classified into diverse subtypes. Although each subtype is known to have different physiological properties, how each subtype contributes to behavior in vivo remains elusive. Detailed analysis on the outcome of depleting MeCP2 from specific interneurons will provide insight into their contributions to specific behaviors. To examine how interneuron subtypes are involved in RTT pathogenesis, we focused on parvalbumin-positive (PV+) and somatostatin-positive (SOM+) neurons, each of which comprise about one third of cortical inhibitory neurons. Using Cre-loxP technology, we generated mice lacking MeCP2 specifically in PV+ cells (PV-conditional knock outs: PV-CKOs) and SOM+ cells (SOM-CKOs). The mice were characterized by multiple behavioral assays, and the results were compared between each CKO and its littermate control groups. We found that PV-CKOs have stronger phenotypes in multiple behavior assays, whereas SOM-CKOs have less but distinct phenotypes that were not present in PV-CKOs. Phenotypes specific to PV-CKOs were motor dysfunction, rigidity, reduced acoustic startle response, and impaired cued memory. Phenotypes specific to SOM-CKOs were epileptic seizure and an increase in compulsive behavior that was measured by hole board assay. Interestingly, both PV and SOM-CKOs had reduced life span. When combined together, these behavioral phenotypes observed in PV and SOM-CKOs covered the majority of phenotypes observed in mice lacking MeCP2 in all inhibitory neurons. Our findings imply differential roles of PV+ and SOM+ cells in the pathogenesis of RTT. The data will provide an important framework for functional studies that will further characterize these inhibitory neuronal subtypes.

Disclosures: **A. Ito-Ishida:** None. **K. Ure:** None. **H.Y. Zoghbi:** None.

Nanosymposium

11. Autism Synaptic and Cellular Mechanisms

Location: 144A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 11.07

Topic: C.06. Developmental Disorders

Support: MRC

Title: The autism and schizophrenia associated gene CYFIP1 is critical for the maintenance of dendritic complexity and the stability of mature spines

Authors: *E. C. DAVENPORT, M. PATHANIA, J. MUIR, D. F. SHEEHAN, G. LÓPEZ-DOMÉNECH, J. T. KITTLER;
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Abstract: Copy number variation (CNV) at the 15q11.2 region of the genome has been identified as a significant risk locus for neuropsychiatric disorders such as autism spectrum disorder (ASD), schizophrenia (SCZ), bipolar disorder and intellectual disability (ID). Investigating the cellular function of genes within this region is important to understand how neuropsychiatric disorders develop. *Cyfip1* is a gene within this region that has been implicated in the pathogenesis of ASD and SCZ. Therefore, understanding how CNV or mutations in *Cyfip1* impair nervous system development, function and connectivity resulting in these disorders is an important goal. Precise control of actin dynamics is critical for the correct development and maintenance of neuronal networks, dendritic arbours and actin-rich spines. *Cyfip1* encodes an essential component of the WAVE regulatory complex (WRC), a complex vital for actin regulation. CYFIP1 maintains the WRC in an inhibited state until the small GTPase Rac1, once activated, binds CYFIP1. This interaction results in CYFIP1 dissociating from the WRC allowing the complex to trigger actin assembly. Here we investigate how *Cyfip1* expression level and mutations in *Cyfip1* impact its actin regulatory function and its role in the regulation of neuronal morphogenesis and synaptic maintenance using mouse genetics, overexpression and imaging techniques. We show CYFIP1 is highly enriched at synapses and its overexpression leads to increased dendritic complexity. Neurons derived from *Cyfip1*$^{+/-}$ animals on the other hand possess reduced dendritic complexity, increased mobile F-actin and enhanced GluA2-containing AMPA receptor mobility at synapses. Interestingly, both *Cyfip1* overexpression and haploinsufficiency increased immature spine number. Thus, CYFIP1 dysregulation leads to pathological changes in CNS maturation and neuronal connectivity. Using similar approaches we investigate how disease associated mutations in *Cyfip1* influence its function. Taken together our findings provide new insights into how genetic alterations in *Cyfip1* may contribute to the development of the neurological symptoms seen in ASD, SCZ and ID.

Disclosures: E.C. Davenport: None. **M. Pathania:** None. **J. Muir:** None. **D.F. Sheehan:** None. **G. López-Doménech:** None. **J.T. Kittler:** None.

Nanosymposium

11. Autism Synaptic and Cellular Mechanisms

Location: 144A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 11.08

Topic: C.06. Developmental Disorders

Support: NIH Grant R01-NS065027

IRSF Postdoctoral Fellowship IRSF-2824

Title: Excitatory CA3->CA1 synapses are stronger in Mecp2 knockout mice and saturate long-term potentiation

Authors: *W. LI, L. POZZO-MILLER;
Neurobio., The Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Unbalanced excitatory and inhibitory synaptic function leading to neuronal network instability has emerged as a common feature in disorders associated with intellectual disabilities, including Rett syndrome (RTT). Using the Mecp2 knockout mouse model of RTT, we found that excitatory synapses onto CA1 pyramidal neurons in hippocampal slices from symptomatic mice have all the hallmarks of "already potentiated" synapses compared to WT neurons: miniature EPSCs in TTX are larger, the slope of input-output curves of evoked EPSCs is steeper, the ratio of AMPAR/NMDAR EPSCs is larger, and membrane currents evoked by bath-applied AMPA are larger. In addition to those biophysical hallmarks of potentiated synapses, the expression levels of GluA1 subunits are higher in Mecp2 knockout mice, as determined by Western immunoblots and semi-quantitative confocal immunohistochemistry. Consistent with this evidence of stronger synapses, either theta-burst stimulation (TBS) of CA3 afferent fibers, or low-frequency afferent stimulation during postsynaptic depolarization failed to potentiate whole-cell EPSCs (and change the A/N ratio), field EPSPs, and the amplitude and spatio-temporal spread of voltage-sensitive dye signals in CA1 of Mecp2 knockout mice. Furthermore, dendritic spines of Mecp2 knockout CA1 pyramidal neurons had larger volumes than spines in wildtype neurons, and did not show the characteristic increase in volume after LTP induction, as determined by 3D reconstructions from confocal z-stacks of biocytin-filled cells during whole-cell recordings. We next examined trafficking of GluA1-containing AMPARs and found that cultured Mecp2 knockout hippocampal neurons had a higher surface-to-total GluA1 ratio than wildtype cells, and failed to show the increase in surface GluA1 after glycine-induced LTP. Similarly, time-lapse imaging of SEP-GluA1 demonstrated that GluA1 intensity did not increase in naïve Mecp2 knockout neurons after glycine-induced chemical LTP, like it did in wildtype cells, indicating impaired surface delivery of pH-sensitive GluA1 into synapses of Mecp2 knockout neurons. We are currently investigating the molecular bases of impaired AMPAR trafficking into and out of hippocampal synapses during activity-dependent plasticity. Collectively, our findings provide molecular, cellular, and network mechanisms of impaired

synaptic transmission and plasticity in Mecp2 knockout mice, which will aid to the rational design and pre-clinical evaluation of novel therapies for RTT.

Disclosures: **W. Li:** None. **L. Pozzo-Miller:** None.

Nanosymposium

16. Spatial and Feature-Based Attention

Location: 146C

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 16.01

Topic: D.04. Vision

Support: NIH Grant EY022727

Title: Distinguishing motor vs. sensory based account of evidence accumulation during perceptual decision making

Authors: ***T. LIU**, M. JIGO;
Michigan State Univ., East Lansing, MI

Abstract: Influential models of decision making postulate a key process that accumulates evidence to threshold, resulting in choice. Previous research has identified a network of frontoparietal areas as possible sites for the neural implementation of evidence accumulation during perceptual decision making. However, the nature of the accumulated evidence is not clear. Here we consider two broad possibilities: evidence for stimulus feature or evidence for motor action. We used the classic random dot motion (RDM) direction discrimination task in an fMRI experiment to test whether the intraparietal sulcus (IPS), a key area implicated by previous studies, accumulates neural evidence for stimulus feature or motor response. Subjects performed three tasks in separate scanning runs. In the first task, subjects performed the standard RDM task and reported the direction of motion (left vs. right) with a saccadic eye movement toward a peripheral target. In the second task, they performed a standard memory delayed saccade task to the same peripheral target. In the third task, they performed a selective attention task where they were cued to attend to either leftward or rightward motion in a compound stimulus that contained two dot fields moving in opposite directions (left and right). The latter two tasks served as the "localizer" scans that allow us to characterize each IPS voxel's preference in terms of the motor plan (saccade) or stimulus feature (motion direction). We then examined single voxel's time course in the RDM task for manifestation of evidence accumulation in the fMRI BOLD signal_a higher BOLD signal for low coherence motion than high coherence motion. We found a

correlation in voxels' stimulus preference and their evidence accumulation profile, such that voxels preferring leftward motion (defined by the attention task) also accumulated evidence for leftward motion, and vice versa. However, voxels preferring a particular eye movement (defined by the memory delayed saccade task) did not exhibit significant correlation with their fMRI signature of accumulate evidence. These results suggest that the posterior parietal cortex accumulates evidence for stimulus features instead of motor action plans during perceptual decision making.

Disclosures: **T. Liu:** None. **M. Jigo:** None.

Nanosymposium

16. Spatial and Feature-Based Attention

Location: 146C

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 16.02

Topic: D.04. Vision

Support: NIH F30 MH097428

NIH CRCNS MH092729

NSF CAREER Award

Title: The impact of categorization training on visual and cognitive encoding in parietal cortex

Authors: *A. SARMA[1], R. KHARKAR[1], X.-J. WANG[2], D. J. FREEDMAN[1];
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Abstract: Recent studies have shown that neuronal activity in the lateral intraparietal (LIP) area can reflect the learned category membership of visual stimuli. However, little is known about how category representations in LIP develop during learning. The primary goal of this project is to understand how category learning influences neuronal encoding in LIP by recording from LIP before, during, and after categorization training. Two monkeys were initially trained to perform a delayed match to sample (DMS) task using 360° of motion directions as sample and test stimuli. Monkeys released a lever to indicate whether a test stimulus showed the same motion direction as a previously presented sample stimulus. 8 sample directions were used during neuronal recordings, and the monkeys correctly identified matching stimuli and +/-75° non-matching stimuli with >90% accuracy. Monkeys were then trained on a delayed match to category (DMC)

task (using the same stimuli) in which they indicated whether sample and test stimuli were in the same category, defined by an arbitrary category boundary. We recorded LIP neurons during the DMS task (N=168), partially trained DMC task (N=171), and fully trained DMC task (N=124). A majority of neurons were direction selective (one-way ANOVA on 8 sample directions, $P<0.01$) during the DMS (110/168), partially trained DMC (88/110) and fully trained DMC (86/124) sample periods. While prior studies in LIP during the DMC task found strong, and often sustained, category selectivity during the memory delay, few cells were direction selective during the DMS delay period (24/168). After categorization training, a larger proportion (Pearson's chi-squared test, $P<0.01$) of cells were direction selective during the DMC delay period (51/124). Delay period category selectivity only appeared in the final DMC stage. In addition to increased delay period selectivity, delay period firing rates were greater during the DMC task compared to the DMS task (t-test, $P<0.01$), indicating increased persistent delay activity in LIP after categorization training. LIP encoded sample direction most strongly during the sample period and sample category most strongly during the delay period. This suggests that learning the DMC task significantly increases direction and category selectivity in LIP during the memory delay period, and that delay period activity and stimulus selectivity in LIP vary markedly depending on task demands and training history. This raises the possibility that more abstract or demanding tasks could enhance persistent activity and selectivity in LIP during working memory, while the DMS task could be mediated by mechanisms other than persistent activity.

Disclosures: **A. Sarma:** None. **D.J. Freedman:** None. **R. Kharkar:** None. **X. Wang:** None.

Nanosymposium

16. Spatial and Feature-Based Attention

Location: 146C

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 16.03

Topic: D.04. Vision

Support: NSF Grant SMA-0835976

NIH Grant R01EY022229

NIH Grant 1F31MH101963

Title: Auditory short-term memory for space but not for time recruits anterior visuotopic parietal maps

Authors: *S. W. MICHALKA[1], M. L. ROSEN[2], L. KONG[2], B. G. SHINN-CUNNINGHAM[1,3], D. C. SOMERS[2];
[1]Ctr. for Computat. Neurosci. and Neural Technol., [2]Psychology, [3]Biomed. Engin., Boston Univ., Boston, MA

Abstract: Audition and vision both convey spatial information about the environment, but much less is known about mechanisms of auditory spatial cognition than visual spatial cognition. Notably, no cortical auditory spatial map representations have been reported; in contrast, more than twenty cortical visuospatial map representations exist, including several near the intraparietal sulcus (IPS) that support visuospatial attention and short-term memory (STM). How does the auditory system keep track of complex spatial information? We employed functional magnetic resonance imaging (fMRI) to investigate this question in six visuotopic regions (IPS0-4, SPL1) and four neighboring non-visuotopic regions in the parietal lobe. Subjects (n = 9) performed an auditory spatial STM task and an auditory timing STM task that employed identical stimuli. The anterior visuotopic parietal maps (IPS2-4, SPL1) were significantly activated in the auditory spatial STM task, but not the auditory timing task. This result could not be attributed to task difficulty or eye movements. Neither auditory task recruited the posterior regions, IPS0 and IPS1, which appear to be exclusively visual. Two non-visuotopic regions (latIPS, antIPS) were driven by both forms of auditory tasks. These results demonstrate that anterior parietal maps are recruited under high auditory spatial demands, while neighboring anterolateral IPS exhibits multi-sensory responses even for non-spatial tasks, and posterior IPS appears to be sensory specific to vision. These findings have important implications for understanding the mechanisms of multi-sensory spatial processing within posterior parietal cortex.

Disclosures: S.W. Michalka: None. **M.L. Rosen:** None. **L. Kong:** None. **B.G. Shinn-Cunningham:** None. **D.C. Somers:** None.

Nanosymposium

16. Spatial and Feature-Based Attention

Location: 146C

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 16.04

Topic: D.04. Vision

Support: NIH R01 EY019041

Title: Interaction between spatial and feature-based attention in parietal cortex

Authors: *G. IBOS, D. J. FREEDMAN;
The Univ. of Chicago, Chicago, IL

Abstract: Feature-based attention (FBA) is the process by which behaviorally relevant stimulus features are selected. In extra-striate visual areas (e.g. MT and V4), FBA modulates the gain of feature selective neurons. Recently we showed that FBA shifts the feature tuning of neurons of the lateral intraparietal area (LIP) toward task-relevant feature values. We posited that this effect is due to the linear integration of inputs from upstream visual areas (e.g. MT and V4). Here, we examine interactions between spatial and feature attention to determine whether spatial attention affects feature integration in LIP. One monkey was trained to perform a delayed conjunction matching (DCM) task in which he had to indicate whether test stimuli matched a previously presented sample while ignoring distractors simultaneously presented in the opposite hemifield. Sample, test and distractor stimuli were high contrast random-dot motion patterns that parametrically varied in both their direction (among 8 evenly spaced directions) and color (8 colors from red to yellow). On each trial, a sample stimulus was shown that was one of two opposite directions (90° or 270°), either red or yellow in color and placed either inside or outside the receptive field (RF) of the neuron being recorded. The monkey was rewarded for responding (with a lever release) to test stimuli that matched the sample in their direction, color and position. Sample and test stimuli were presented either inside the RF (distractors presented outside the RF) or in the opposite hemifield (distractors presented inside the RF). This allowed us to test whether neuronal color and direction tuning depended on which feature-values and spatial position were behaviorally relevant. We recorded from 17 LIP neurons while one monkey performed the DCM task. We found that spatial attention dramatically modulated the visual feature representations in LIP. When the monkey was attending inside the neurons' RFs, 13/17 LIP neurons were direction selective and 14/17 were color selective (3-way ANOVA with direction, color and sample identity as factors, $p<0.01$). 3/17 and 13/17 LIP neurons showed significant shifts of direction and color tuning, respectively, toward the relevant feature-values (permutation test, $p<0.05$). In contrast, when attending outside the RF, only 4/17 neurons were direction selective and 1/17 neuron was color selective. In this case, the direction and color tuning of only 1/17 and 2/17 LIP neurons were significantly shifted toward the relevant feature-values These results suggest that top-down spatial attention gates the bottom-up integration of non-spatial visual information by LIP neurons.

Disclosures: G. Ibos: None. **D.J. Freedman:** None.

Nanosymposium

16. Spatial and Feature-Based Attention

Location: 146C

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 16.05

Topic: D.04. Vision

Support: NIH grant 1R01EY022355 to Y.X.

Title: Decoding invariant visual object representations in human parietal cortex

Authors: *M. VAZIRI PASHKAM;
Vision Scinces Laboratory, Dept. of Psychology, Harvard Univ., Cambridge, MA

Abstract: Although visual object representations have been discovered in the primate parietal cortex more than a decade ago, the nature of these representations remains largely unknown. Using fMRI and multiple voxel pattern analysis, we investigated the represetnation of objects from eight different categories in human parietal cortex. The parietal regions we examined included five topographic areas along the intra-parietal sulcus (IPS) as well as superior and inferior IPS, two regions previously implicated in visual object individuation and identification, respectively. We also examined responses from retinotopically defined early visual areas, the object shape processing region in lateral occipital cortex and part of temporal cortex activated by our object stimuli. Using a linear support vector machine classifier, we obtained significant decoding of the different object categories in both occipito-temporal and parietal regions. In particular, successful decoding was observed across changes in position and size in both occipito-temporal and parietal regions, but not in early visual cortex. Moreover, in both occipito-temporal and parietal regions, diverting attention away from the object location decreased category decoding performance; whereas diverting attention away from the category-relevant feature (i.e., shape) to a category-irrelevant feature (e.g., color) of the same object did not impact decoding performance. We also constructed an object similarity matrix for each brain region from the decoding results to examine representation similarity between the different object categories in that region. We then correlated the similarity matrices from different brain regions and found that object category representations are highly similar between superior IPS and occitpio-temporal regions. These results demonstrate that objects from different categories can be represented distinctively in human parietal cortex. These representations show both position and size invariance and are modulated by spatial attention. Moreover, object representations in human parietal cortex are similar to those in occipital and temporal cortices, and likely reflect high levels of visual object processing.

Disclosures: M. Vaziri Pashkam: None.

Nanosymposium

16. Spatial and Feature-Based Attention

Location: 146C

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 16.06

Topic: D.04. Vision

Support: NIH Grant MH099611

NIH Grant EY017366

CRCNS

McKnight Foundation

Title: Choice probabilities and correlations in simultaneously recorded MT and LIP neurons

Authors: *J. YATES[1,2], L. N. KATZ[2], I. PARK[2], J. W. PILLOW[2], A. C. HUK[2];
[1]Inst. for Neurosci., Univ. of Texas At Austin, Austin, TX; [2]The Univ. of Texas at Austin, Austin, TX

Abstract: Two decades of recordings in posterior parietal cortex of primates suggests that neurons in the lateral intraparietal area (LIP) integrate noisy sensory evidence from neurons in middle temporal area (MT) to make decisions about visual motion. Relatively little is known, however, about the flow of signal and noise between these areas at the scale of single trials, and across neuronal populations. Here we recorded from multiple cells in MT and LIP (2-15 simultaneously) while macaques performed a reverse correlation direction-discrimination task. The subject discriminated the net direction of motion in a dynamic noise stimulus and indicated his choice with a saccade to one of two targets. One target was placed in the response fields of a cluster of LIP cells, and the motion stimulus was placed in the receptive fields of a cluster of MT cells. The motion stimulus consisted of a patch of small flickering gabors, some of which pulsed in either of the target directions at different times throughout the trial. The pulses were statistically independent to measure the subject's temporal weighting of the stimulus with reverse correlation. On average, early pulses contributed most to the monkey's choice (27 sessions). Additionally, we computed choice probability (CP) in MT (n=45) and LIP (n=71) cells and compared the CP to the subject's temporal weighting. Average CP in MT was slightly above .5 (.53), decreased throughout stimulus viewing, and correlated with the temporal weighting. CP in LIP evolved later during stimulus viewing to a higher value (.62), and was inversely correlated with the temporal weighting. We used a generalized linear model (GLM) to fit single trial spike trains and characterized the response selectivities of MT and LIP cells to different stimulus

components (target onset, motion direction, reward). The GLM included each cell's own spike history as well as inter-neuronal coupling between simultaneously recorded cells. Cell pairs in both MT (n=80) and LIP (n=117) exhibited short and long timescale coupling that could not be explained by common stimulus drive. However, we found no evidence of coupling between MT and LIP (n=142). These data suggest that the choice-related activity in LIP may not be driven directly from MT inputs.

Disclosures: **J. Yates:** None. **L.N. Katz:** None. **I. Park:** None. **J.W. Pillow:** None. **A.C. Huk:** None.

Nanosymposium

16. Spatial and Feature-Based Attention

Location: 146C

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 16.07

Topic: D.04. Vision

Title: Attention signals based on object category selection in frontoparietal cortex

Authors: ***K. N. SEIDL**[1,2], J. G. KIM[2], M. V. PEELEN[3], S. KASTNER[1,2];
[1]Psychology Department, Princeton Univ., Princeton, NJ; [2]Princeton Neurosci. Institute, Princeton Univ., Princeton, NJ; [3]Ctr. of Mind/Brain Sciences, Univ. of Trento, Trento, Italy

Abstract: A distributed network of topographically organized areas in frontal and parietal cortex has consistently been implicated in the exertion of space-based and feature-based attentional control. Here we investigate the role of these regions in the control of category-based attentional biasing signals that mediate real-world visual search. Topographic regions in posterior parietal cortex (PPC) in particular appear to be well suited for the control of category-based attention signals: (i) a subset of PPC regions carry object-selective responses similar to those observed in ventral object-selective regions (OSC); (ii) PPC plays a prominent role in working memory, making it well suited for the maintenance of category-based attentional sets; and (iii) PPC has strong anatomical connections with OSC where category-specific attentional sets are implemented. Here, we used fMRI to investigate object category-based and space-based attention signals in topographic areas of the frontoparietal network and OSC. Participants were briefly presented with natural scenes to the left and right of a central fixation cross. On separate runs, participants spatially attended to either the left or the right scene. A central cue at the beginning of each trial instructed participants to detect either people or cars in the spatially attended scene. The extent to which category or space information was present in scene-evoked

activation patterns was estimated by testing how similar they were to the responses evoked by isolated category exemplars shown to the left or right of fixation during separate scans. Consistent with the role of the frontoparietal network in spatial attention, response patterns in these regions carried information about which scene subjects were spatially attending to. Patterns in OSC also carried significant information for the direction of spatial attention. In line with previous findings, OSC represented the currently relevant category more strongly than the irrelevant one. This was true both for spatially attended and unattended scenes. Response patterns evoked by spatially attended scenes carried overall higher category information suggesting that space-based attention can result in a general boost of the strength of object representations in OSC. Posterior IPS but not anterior IPS also carried more information for the currently relevant compared to the irrelevant category. This effect appeared to be more pronounced in the spatially attended scenes. Thus, at least a subset of areas within the frontoparietal attention network may be involved in the control of category-based attention in addition to space-based and feature-based attention.

Disclosures: **K.N. Seidl:** None. **J.G. Kim:** None. **M.V. Peelen:** None. **S. Kastner:** None.

Nanosymposium

16. Spatial and Feature-Based Attention

Location: 146C

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 16.08

Topic: D.04. Vision

Support: NIH grant EY022355

Title: Abstract face identity representation in human superior intra-parietal sulcus reflects perceived face identity similarity

Authors: ***S. JEONG**, Y. XU;
Dept. of Psychology, Harvard Univ., Cambridge, MA

Abstract: Emerging evidence from both neurophysiology and human brain imaging suggests that, in addition to exerting top down attentional control over sensory areas, the primate parietal cortex is capable of directly representing task-driven visual information. The human superior intra-parietal sulcus (IPS), in particular, has been shown to track the amount of information encoded in visual short-term memory in a task-dependent manner. Supporting this finding, in previous studies using fMRI MVPA, we obtained successful decoding of task-relevant visual

information in superior IPS. Decoding was not only obtained for basic-level visual features such as object shapes, but also for high-level abstract visual representations such as viewpoint invariant face and car identities, indicating that superior IPS is capable of representing a great variety of visual information. Nevertheless, as identity co-varies with a host of other factors, such as familiarity and attractiveness, it is unclear whether decoding in superior IPS reflects identity representation per se, or these other factors. In this study, we correlated neural and behavioral identity similarity measures to address this question. Participants viewed face images from eight well-known actors, with the images for each actor varied in viewpoint, hairstyle, and facial expressions. Replicating our previous results, in superior IPS, we found fMRI response patterns for face images belonging to the same actor to be more correlated than those for face images from different actors, indicating the encoding of face identity in superior IPS. Such identity representation was not found in other brain regions such as the lateral occipital (LO) region, the right fusiform face area (FFA), and the lateral prefrontal cortex. We then constructed a face identity similarity matrix by computing the correlation between fMRI response patterns evoked by face images from each pair of actors. To obtain behavioral face identity similarity measure, using the same face stimuli, we asked participants to search for the face of a target actor embedded among the faces of a distractor actor. Using the search response time, we constructed a behavioral face identity similarity matrix. We found significant correlation of neural and behavioral face identity similarity matrix in superior IPS, but not in LO or the right FFA. Thus representations contained in superior IPS reflect perceived face identity similarity. This result provides a critical piece of evidence showing a strong involvement of superior IPS in the moment-to-moment goal-directed visual information representation in the human brain.

Disclosures: S. Jeong: None. **Y. Xu:** None.

Nanosymposium

16. Spatial and Feature-Based Attention

Location: 146C

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 16.09

Topic: D.04. Vision

Support: 1R01MH098039-01

Title: Reward and uncertainty shape oculomotor behavior in non-human primates

Authors: *N. DADDAOUA[1], J. GOTTLIEB[2];
[1]1051 Riverside Drive, Kolb Annex, Unit 87, New York, NY; [2]Dept. of Neurosci., Columbia Univ., New York, NY

Abstract: Over the past years there has been increased interest in the neural bases of information seeking and its role in decision making and selective attention. Prior evidence suggests that oculomotor information sampling during natural tasks is based on uncertainty and reward and that the parietal lobe is important for uncertainty-based behaviors, but the neural correlates of these processes are not understood. To study this question we are developing behavioral paradigms that can be used in monkeys and humans and can effectively isolate the roles of uncertainty and reward in oculomotor decisions. We used a task where monkeys viewed two sequential cues that signaled the expected reward of the trial, such that the information brought by the second cue was either new or redundant relative to the first. On redundant-cue trials the first cue signaled a 100% or 0% likelihood of reward and the second cue merely confirmed these predictions. By contrast, on informative-cue trials, the first cue signaled a 50% (uncertain) outcome, and the second cue specified whether the trial had a 100% or 0% likelihood of reward. In two monkeys, saccades to the second cue were strongly dependent on expected reward and showed a smaller effect of new information. The likelihood of making a saccade and the post-saccadic dwell times were much higher if the cues signaled a 100% relative to a 0% likelihood, indicating a strong effect of reward. Importantly, because the cues were merely informational, this effect is due to Pavlovian cue-reward associations rather than an active strategy for increasing reward by examining the cue. In some sessions, dwell times on the second cues showed a larger reward effect when the cue was informative than when it was redundant, revealing an additional effect of uncertainty reduction. In a second paradigm we examined whether the monkeys actively forage for information. We presented a first cue signaling 100%, 50% or 0% reward followed by a display with 3 opaque placeholders. A randomly selected placeholder was hiding the second cue and the monkeys could reveal it by making a saccade. Exploratory behavior was higher after a 100% than 0% first cues, revealing a reward effect, and was also higher after a 50% than a 100% cue, revealing an effect of uncertainty. The results reveal the interacting roles of uncertainty and reward in gaze behavior in a task that is suitable for neural recordings in monkeys.

Disclosures: N. Daddaoua: None. **J. Gottlieb:** None.

Nanosymposium

16. Spatial and Feature-Based Attention

Location: 146C

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 16.10

Topic: D.04. Vision

Support: NIH R01EY022229

NSF SMA-0835976

Title: Memory-guided attention and stimulus-guided attention networks in human parietal lobe

Authors: *M. L. ROSEN[1], C. E. STERN[1,2], S. W. MICHALKA[3], K. J. DEVANEY[1], L. KONG[1], D. C. SOMERS[1,2,3];
[1]Dept. of Psychological and Brain Sci., [2]Grad. Program for Neurosci., [3]Ctr. for Computat. Neurosci. and Neural Technol., Boston Univ., Boston, MA

Abstract: Human visual abilities exceed that of powerful supercomputers and yet our attentional capacity is limited to approximately four items. We can reconcile this apparent paradox by considering the extensive capacity of long-term memory (LTM). Prior experience can guide attention and boost performance. Here, we investigated long-term memory guidance of visual attention and stimulus-guided attention using a change detection task. Participants studied changes in 24 real-world visual scenes and viewed another set of images with no changes. The following day, while undergoing fMRI scanning, subjects performed a one-shot change detection task under LTM-guided or stimulus-guided conditions. Three posterior regions were significantly more strongly recruited for LTM-guided attention than stimulus-guided attention: the mid-cingulate/posterior callosal sulcus, the posterior precuneus, and the lateral intraparietal sulcus. A recent meta-analysis (Power *et* al., 2011) suggested that these three regions, the mid-cingulate, posterior precuneus and lateral intraparietal sulcus, make up a memory retrieval network; our findings support this hypothesis. Analysis of activation patterns within visuotopic parietal regions of the dorsal attention network also revealed differences between long-term LTM-guided attention and stimulus guided attention. Previous studies have shown that under high attentional or visual short term memory load (Sheremata *et al*., 2010; Szczepanski *et al*., 2010), the left visuotopic IPS responds to targets in the right (contralateral) visual field, whereas the right visuotopic IPS responds to targets in both the left (contralateral) and right (ipsilateral) visual field; these results suggest that the spatial representations of the right hemisphere IPS regions dynamically shift between stimulus driven and attention/STM conditions. Results from the current stimulus-guided condition replicate this prior hemispheric asymmetry using complex real world scenes. In the LTM-guided condition, both the right and left visuotopic IPS exhibited strong bilateral responses. Thus hemispheric symmetry is observed in IPS in the LTM-guided condition, but the responses exhibit much less contralateral specificity than observed for retinotopic mapping. These findings suggest that attentional demands drive right IPS, while long-term memory retrieval demands drive left IPS. Taken together, our findings help to further our understanding of long-term memory-guided visual attention.

Disclosures: **M.L. Rosen:** None. **C.E. Stern:** None. **S.W. Michalka:** None. **K.J. Devaney:** None. **L. Kong:** None. **D.C. Somers:** None.

Nanosymposium

16. Spatial and Feature-Based Attention

Location: 146C

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 16.11

Topic: D.04. Vision

Support: NIH Grant P30-NS076408

NIH Grant R01-EY014989

NIH Grant T32-GM8471-19

NIH Grant T32-GM8244-23

NIH Grant T32-HD0071-51

NIH Grant P41-RR0080-79

NIH Grant S10-RR0267-83

Title: Non-spatial attention selectively biases orientation tuning in human V1

Authors: *S. G. WARREN[1], E. S. YACOUB[2], G. M. GHOSE[1];
[1]Dept. of Neurosci., [2]Ctr. for Magnetic Resonance Res., Univ. of Minnesota, Minneapolis, MN

Abstract: Many studies have demonstrated that spatial attention modulates activity at multiple stages of cortical visual processing by enhancing the responses of neurons which encode the attended region of space. However, we often allocate attention not solely on the basis of location, but also according to behaviorally relevant attributes or timing. To better understand the physiological changes underlying such non-spatial attention, we collected BOLD functional images at 7T from primary visual cortex of nine human subjects while they performed a feature- and time-based change detection task. Subjects were required to detect brief spatial frequency changes in a large-field and continuously rotating grating and were cued prior to the rotation as to the orientation for which changes were likely. By looking for voxels that were modulated by this rotation, we found that many single voxels (14-32%) were well-tuned to the orientation of the grating. Cue presentation systematically biased both subject reaction times and the

orientation tuning of individual voxels. This attentional change was well-described by a linear model in which modulations were greatest in voxels whose preferred orientation was aligned with the cue and at times when the cue was most behaviorally relevant. This model is consistent with previous results from both primate and human electrophysiology, and suggests that all modalities of visual attention act by selectively enhancing the most task-appropriate neurons during appropriate periods of time.

Disclosures: S.G. Warren: None. **E.S. Yacoub:** None. **G.M. Ghose:** None.

Nanosymposium

16. Spatial and Feature-Based Attention

Location: 146C

Time: Saturday, November 15, 2014, 1:00 PM - 4:00 PM

Presentation Number: 16.12

Topic: D.04. Vision

Title: Neuronal activity in the superior colliculus during a color-change task

Authors: *J. P. HERMAN, R. KRAUZLIS;
Lab. of Sensorimotor Res., Bethesda, MD

Abstract: The primate superior colliculus (SC) is one of three brain areas with a "priority map". Along with the frontal eye field (FEF) and lateral intraparietal area (LIP), SC neurons signal the location of task-relevant stimuli and participate in the control of covert spatial attention. However, studies of covert spatial attention have mostly used motion and orientation stimuli. In order to understand how broadly the SC participates in the control of spatial attention, we examined responses of SC neurons during a color change task. We recorded from units in the superficial and intermediate layers of the SC (n = 54) while a monkey performed a covert attention task employing dynamic color "checkerboard" stimuli. Our color stimuli were partially inspired by random dot stimuli; checks were presented for a fixed lifetime in frames, and each square's color was then redrawn from a Gaussian distribution on an isoluminant DKL color plane with an added luminance perturbation. While fixating centrally and pressing a joystick, the animal was presented with two spatially opposed color patches (3.25° radius, ~10° eccentricity), one of which had been cued by a briefly flashed ring. The animal's task was to release the joystick if the cued patch's mean color changed, and ignore changes in the opposing foil by maintaining joystick press. These color changes were physically isoluminant increases in saturation, masked by luminance noise, and adjusted to maintain performance near 80% correct. In addition to an onset transient and steady-state response to the color stimulus, the most notable

feature of single unit responses was a vigorous increase in activity triggered by the color changes. This change-related activity was present in the majority of units (50/54), was quite rapid, becoming significant within 90-150ms, and in most cases (30/50) was larger than the activity elicited by stimulus onset. Modulation due to cueing was evident throughout the response to the stimulus, including the change-related activity, which rose more rapidly when evoked by a cued stimulus change (112ms vs. 138ms). Additionally, the amplitude of this change-related activity was predictive of the animal's behavioral response. Our results demonstrate that the SC has access to color information and participates in the discrimination of near-threshold color stimuli. These results support the view that the SC may contribute to the processing of any behaviorally relevant visual event, regardless of feature dimension.

Disclosures: J.P. Herman: None. **R. Krauzlis:** None.

Nanosymposium

17. Plasticity After Spinal Cord Injury

Location: 143A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 17.01

Topic: D.10. Spinal Cord Injury and Plasticity

Support: INSERM

Title: The regulation of a type-4 desintegrin and metalloproteinase with thrombospondin motifs (ADAMTS-4) by tissue-type plasminogen activator (tPA) in the central nervous system

Authors: *M. PRUVOST[1], E. MAUBERT[1], S. LEMARCHANT[2], E. EMERY[1,3], F. DOCAGNE[1], D. VIVIEN[1];
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Abstract: Objectives Tissue-type plasminogen activator (tPA) is a serine protease that regulates physiological processes in the brain such as learning and memory, and plays a critical role in neuronal survival and neuroinflammation in pathological conditions. Recent studies in our lab highlighted a new target for tPA: a type 4 disintegrin and metalloproteinase with thrombospondin motifs motifs (ADAMTS-4). ADAMTS-4 is known to degrade chondroitin suflate proteoglycans (CSPGs). CSPGs play critical role in the organization of the brain extracellular matrix thus influencing neuronal growth and plasticity. CSPGs are dramatically

upregulated after central nervous system (CNS) injury within the glial scar. The aim of the present study was to further investigate the relationships between tPA and ADAMTS-4. Materials and methods Mice protein extracts were incubated with recombinant tPA, an inactive tPA (GGACK-tPA) or Plasmin. The endogenous activity of ADAMTS-4 was measured using the SensoLyte® 520 Aggrecanase-1 assay kit. To characterize the effect of endogenous tPA on ADAMTS-4 expression we produced a new strain of double tPAKO/ADAMTS-4 KO/lacZ transgenic mice. Immunohistochemistry was performed on mouse brain and spinal cord sections. Results The activity of endogenous ADAMTS-4 is increased in spinal cord protein extracts incubated with growing doses of tPA. This increased activity of ADAMTS-4 was not observed with the inactive tPA (GGACK-tPA) or plasmin, suggesting that the activation of pro-ADAMTS-4 into its mature form by tPA occurs through a plasmin(ogen)-independent mechanism that requires its proteolytic activity. Immunohistochemistry performed on double transgenic mice revealed different patterns of expression of the tPA/ADAMTS-4 axis in these mice. Conclusion tPA has multiple effects in the CNS. Some of its effects could be due to its newly described target: ADAMTS-4. There is growing evidence that ADAMTS-4 plays important roles in the CNS because of its ability to degrade inhibitory molecules involved in neuronal plasticity and regeneration. In this study, we provide new information on the transcriptional and translational mechanisms underlying the tPA ADAMTS-4 axis.

Disclosures: **M. Pruvost:** None. **E. Maubert:** None. **S. Lemarchant:** None. **E. Emery:** None. **F. Docagne:** None. **D. Vivien:** None.

Nanosymposium

17. Plasticity After Spinal Cord Injury

Location: 143A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 17.02

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH R21 NS087320-01

The Grainger Foundation

Title: Imaged-guided, stereotactic delivery of intraspinal stimulating electrodes to restore function following spinal cord injury

Authors: ***P. GRAHN**[1], G. W. MALLORY[2], S. J. GOERSS[2], J. JEONG[2], D. A. LOBEL[6], A. J. BIEBER[2,3], K. E. BENNET[2,4], J. L. LUJAN[2,5], K. H. LEE[2,5];

[1]Mayo Grad. Sch., [2]Dept. of Neurologic Surgery, [3]Dept. of Neurol., [4]Div. of Engin., [5]Dept. of Biomed. Engin. and Physiol., Mayo Clin., Rochester, MN; [6]Dept. of Neurosurg., Cleveland Clin. Fndn., Cleveland, OH

Abstract: Intraspinal microstimulation (ISMS) is an emerging technique for restoring function lost to paralysis following spinal cord injury. Multiple animal studies have shown ISMS is capable of controlling of limb movements while improving fatigue resistance. Nevertheless, several limitations have prevented translation of ISMS technology into humans. Perhaps the most significant obstacle to translation is the difficulty of targeting specific spinal cord regions responsible for selective motor function. Current ISMS methods for restoring locomotion involve implantation of multiple stimulating electrodes or electrode arrays, followed by characterization of stimulation-evoked motor responses. However, electrode targeting relies on external anatomical landmarks, and is thus susceptible to targeting errors due to anatomical differences between subjects. Furthermore, there is a large potential for trajectory deviations during electrode insertion due to the mechanical properties of the tissue and inconsistent forces applied during manual electrode insertion. To minimize these targeting errors and thus improve selectivity, we have designed a frame-based magnetic resonance imaging (MRI)-compatible stereotactic targeting and electrode delivery system. The stereotactic system described herein offers the potential for improved targeting and selective stimulation of neuronal populations responsible for specific motor function, thereby reducing the spatial inaccuracy of existing methods. In turn, use of this system will minimize insertion-related trauma by reducing the number of insertion attempts required to successfully implant electrodes into desired locations To test this stereotactic delivery platform, we have established a porcine model of ISMS that offers significant similarities with human anatomy and physiology and is paramount for expedited translation of this technology to human applications. Using this porcine model, we compared the accuracy and precision of delivering microwire electrodes into the spinal cord using either our image-guided spine frame delivery, or conventional anatomical landmark-based manual electrode implantation. Initial application of the stereotactic system is intended for ISMS. However, the targeting and delivery system described herein will have additional therapeutic applications such as fine needle biopsies, and intraspinal delivery of drugs and stem cells.

Disclosures: **P. Grahn:** None. **G.W. Mallory:** None. **S.J. Goerss:** None. **J. Jeong:** None. **D.A. Lobel:** None. **A.J. Bieber:** None. **K.E. Bennet:** None. **K.H. Lee:** None. **J.L. Lujan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Boston Scientific.

Nanosymposium

17. Plasticity After Spinal Cord Injury

Location: 143A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 17.03

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Travis Roy Foundation

Title: Paired motor cortex and cervical spinal cord stimulation augments corticospinal motor responses and promotes learning in the spinal cord of rats

Authors: *A. M. MISHRA[1], D. GUPTA[1], J. B. CARMEL[1,2];
[1]Motor Recovery Laboratory, Burke Med. Res., White Plains, NY; [2]Brain and Mind Res. Inst. and Departments of Neurol. and Pediatrics, Weill Med. Col. of Cornell Univ., New York, NY

Abstract: Spinal epidural stimulation has emerged as a powerful tool to raise the excitability of spinal cord circuits and to strengthen voluntary movement after injury. We sought to augment excitability of the corticospinal tract by pairing stimulation of its origin in motor cortex with stimulation of its terminations in the cervical spinal cord. All of the experiments were conducted in intact, anesthetized adult rats. We assayed excitability of the motor system by stimulating motor cortex with trains of 3 biphasic pulses at 333 Hz using and recording EMG in the contralateral biceps muscle. We created recruitment curves by stimulating cortex at increasing intensity and compared curve under different conditions. We used silver ball electrodes on the dorsum of the cervical spinal cord to deliver epidural stimulation. We conducted 3 experiments. In the first experiment, we measured the effects of tonic 40Hz spinal epidural stimulation on EMG responses. We hypothesized that, like lumber epidural stimulation, tonic cervical spinal cord stimulation would augment EMG responses in a manner dependent on the intensity, polarity, and stimulation location. Indeed, tonic stimulation directed at the cervical enlargement produced robust augmentation of EMG with both cathodal and biphasic stimulation that increased with intensity. In the second experiment, we hypothesized that a single pulse of spinal stimulation at discrete intervals after cortex stimulation would augment EMG responses. Latency was a crucial determinant, with 11ms being optimal. This timing coincides with the timing of the cord dorsum potential recorded in the cervical cord after motor cortex stimulation, suggesting synergistic effects of corticospinal and large diameter afferent stimulation. Finally, we asked whether repeatedly pairing of cortex and spinal cord stimulation at the optimal latency would induce learning in the spinal cord. We created a baseline response curve and also measured the spinal stimulation necessary to provoke EMG responses. We then delivered motor cortex stimulation followed 11ms later by a single biphasic spinal cord pulse and repeated this every 2 seconds for 5 minutes for a total of150 paired stimuli. We recorded a response curve and spinal thresholds immediately after the pairings and every 10 minutes thereafter. Paired stimulation caused a dramatic (>100%) increase in motor responses and the spinal threshold also decreased. Thus, we demonstrate plasticity in the intact CST by repetitive pairing of brain and spinal cord stimulation that occurs at the level of spinal cord.

Disclosures: **A.M. Mishra:** None. **D. Gupta:** None. **J.B. Carmel:** None.

Nanosymposium

17. Plasticity After Spinal Cord Injury

Location: 143A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 17.04

Topic: D.10. Spinal Cord Injury and Plasticity

Title: Similar changes in corticospinal excitability after anodal tDCS between 1 mA and 2 mA

Authors: ***L. M. MURRAY**[1,2], K. NOSAKA[2];
[1]Physical Med. and Rehabil., Hamad Med. Corp., Doha, Qatar; [2]Sch. of Exercise and Hlth. Sci., Edith Cowan Univ., Joondalup, Western Australia, Australia

Abstract: In recent years, there has been a rapid increase in the use of anodal transcranial direct current stimulation (a-tDCS) as a neuromodulation technique. Stimulation intensity is a factor affecting cortical excitability changes, but controversy exists concerning the increases in corticospinal excitability after 1 mA and 2 mA a-tDCS, which are often used. It is assumed that increasing current intensity, whilst keeping other parameters constant, will result in enhanced efficacy of the stimulation, but this has not been systematically investigated. Therefore, the present study was designed to investigate whether doubling a-tDCS intensity would affect its effect on corticospinal excitability. In a randomized, counterbalanced, single-blinded trial, 12 healthy participants (6F, 6M) received three interventions (1 mA, 2 mA or sham tDCS, anodal, 20 minutes, left M1, 35 cm^2 electrodes) in a crossover design (>3 days between sessions). To assess modulations in corticospinal excitability, transcranial magnetic stimulation induced motor evoked potentials (MEPs) of the right extensor carpi radialis (ECR) muscle, were recorded at baseline and every 10 minutes for one-hour after each tDCS intervention. Changes in MEP amplitude over time were compared amongst the three interventions by a two-way repeated measures ANOVA. MEP amplitude was significantly increased by a mean of 41% and maintained for up to 1 hour following 1 mA a-tDCS ($p=0.003$), and 47% for up to 50 min following 2 mA a-TDCS ($p=0.019$). No significant differences were evident in the changes in MEP amplitude responses over time between the two a-tDCS conditions, however both were significantly different to sham (1 mA: $p=0.010$; 2 mA: $p=0.001$). No significant changes in MEP amplitude were observed after sham tDCS. All interventions were well tolerated by the participants, and no adverse effects were observed. These results show that a 20-minute application of a-tDCS transiently increases excitability of the corticospinal projections to the

right ECR muscle following 1 and 2 mA stimulation similarly. Doubling the intensity had no added effects on corticomotor excitability, suggesting that synaptic mechanisms associated with long-term potentiation may not be influenced by stimulation intensity.

Disclosures: L.M. Murray: None. **K. Nosaka:** None.

Nanosymposium

17. Plasticity After Spinal Cord Injury

Location: 143A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 17.05

Topic: D.10. Spinal Cord Injury and Plasticity

Title: Spinal cord maturation and locomotion in mice with an isolated cortex

Authors: *Q. HAN;
GHM Inst. of CNS Regeneration, Jinan Universit, Guangdong, China

Abstract: The spinal cord plays a key role in motor behavior. It relays major sensory information, receives afferents from supraspinal centers and integrates movement in the central pattern generators. Spinal motor output is controlled via corticofugal pathways including corticospinal and cortico-subcortical projections. Spinal cord injury damages descending supraspinal as well as ascending sensory pathways. In adult rodent models, plasticity of the spinal cord is thought to contribute to functional recovery. How much spinal cord function depends on cortical input is not well known. Here, we address this question using Celsr3/Foxg1 mice, in which cortico-subcortical connections (including corticospinal tract (CST) and the terminal sensory pathway, the thalamocortical tract) are genetically ablated during early development. Although Celsr3/Foxg1 mice are able to eat, walk, climb on grids and swim, open-field tests showed them to be hyperactive. When compared with normal littermates, mutant animals had reduced number of spinal motor neurons, with atrophic dendritic trees. Furthermore, motor axon terminals were decreased in number, and this was confirmed by electromyography. The number of cholinergic, calbindin, and calretinin-positive interneurons was moderately increased in the mutant spinal cord, whereas that of reelin and parvalbumin-positive interneurons was unchanged. As far as we know, our study provides the first genetic evidence that the spinal motor network does not mature fully in the absence of corticofugal connections, and that some motor function is preserved despite congenital absence of the CST.

Disclosures: Q. Han: None.

Nanosymposium

17. Plasticity After Spinal Cord Injury

Location: 143A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 17.06

Topic: D.10. Spinal Cord Injury and Plasticity

Support: IBRO-SfN Travel Grant

Title: What do we know about the role of endothelial ETA and ETB receptors on neuropathic pain following spinal cord injury?

Authors: *S. FORNER[1], A. C. MARTINI[2], E. L. DE ANDRADE[2], G. A. RAE[2];
[1]Farmacologia, Univ. Federal De Santa Catarina, Florianopolis, Brazil; [2]Farmacologia, Univ. Federal de Santa Catarina, Florianópolis, Brazil

Abstract: Traumatic spinal cord injury (SCI) is a devastating neurologic disorder. Individuals with SCI often develop neuropathic pain, which has a major impact on their quality of life. This condition is due to functional and structural plastic changes that occur centrally following injury and includes changes in receptor function to increase neuronal excitability. Endothelins are a family of peptides that exert their biological effects via distinct endothelin A (ETAR) and endothelin B (ETBR) receptors and contribute to sensory changes in inflammatory and neuropathic pain. However, their role in nociception following SCI still remains to be elucidated. SCI was induced in adult male Wistar rats by inflating an embolectomy catheter at T10 level. We evaluated the sensitivity of the animals to mechanical (von Frey monofilaments) stimulation of the paws on days 2, 7, 14, 21 and 28 after SCI. e mRNA levels for ETAR and ETBR in the spinal cord and dorsal root ganglion in all periods post surgery, as well as protein expression of both receptors of spinal cord and localization in the spinal cord (white and grey matter) were evaluated. We assessed changes in mechanical sensitivity of hindpaws 21 days post-surgery from 30 min to 270 min after the intrathecal injection of BQ-123 (ETAR antagonist), BQ-788 (ETBR antagonist) and after the oral administration of Bosentan (ETA/ETBR antagonist) from 1 to 6 hours after the treatment. All procedures were approved by the local Ethics Committee (#PP00680). The frequency of responses to mechanical stimulation of forepaws was unchanged at any time point, but that of hind paws was increased at 14, 21 and 28 days. An upregulation of ETAR expression was detected in spinal cord on the 21st after SCI, but ETBR expression was not altered. ETAR mRNA level was increased in the spinal cord on days 7, 14, 21 and 28 days and on DRG on day 7 post-SCI. ETBR mRNA levels were increased in the spinal cord on days 2 and 7 post-surgery, but those in DRG were unchanged at all periods analyzed. There is also an upregulation of ETAR on 14th day post-SCI in the gray matter of the spinal cord, however there

were no changes for ETBR. No changes of both receptors were observed in the white matter after SCI. Treatment with BQ-123 on the 21st day post-SCI reduced mechanical sensitivity of hindpaws measured 150 and 210 min after administration when compared to its vehicle group, while BQ-788 had no changes. Bosentan had a reduction from 2 to 4 hours after the treatment. Taken together, these data show the important roles of ETAR and ETBR in neuropathic pain following SCI in rats and suggest that blockade of these receptors could be a potential therapeutic target for controlling such condition.

Disclosures: S. Forner: None. **A.C. Martini:** None. **E.L. de Andrade:** None. **G.A. Rae:** None.

Nanosymposium

17. Plasticity After Spinal Cord Injury

Location: 143A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 17.07

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Danish Research Council Post Doc Grant DFF-1333-00197

Title: Pathological changes of the triceps Surae muscle in rats 8 weeks following a spinal cord hemisection

Authors: *J. PINGEL, J. LORENTZEN, J. WIENECKE, J. B. NIELSEN;
Dept. of Neurosci. and Pharmacol., Univ. of Copenhagen, Copenhagen, Denmark

Abstract: Pathological changes of the triceps Surae muscle in rats 8 weeks following a spinal cord hemisection Pingel J., Lorentzen J., Wienecke J. and Nielsen JB Department of Exercise and Nutrition & Department of Neuroscience and Pharmacology **Introduction:** Spinal cord injury leads to severe problems involving impaired motor, sensory, and autonomic functions. After a spinal injury there is an initial phase of hyporeflexia followed by hyperreflexia, often referred to as spasticity. Previous studies have suggested that hyperreflexia and overactivity of the muscle is related to the development of muscle contractures. **Aim:** The aim of the present study was to elucidate whether over- or underactivity of the triceps surae muscle leads to increased passive stiffness (contractures) with changes in collagen composition of the muscle in rats. **Participants and Methods:** A hemisection model was combined with immobilization and botox injections in the triceps surae to provoke prolonged over- and underactivity in the muscle. Eight weeks after the intervention both passive stiffness and reflex torque of the muscle, and

changes of the gene expression of several collagens were identified in both hindlimbs. **Results:** Spinal cord hemisection caused a significant increase of triceps surae stretch reflex and reflex-mediated stiffness. No change in passive stiffness was observed in the muscle 2 month and 6 month after the lesion. Botox injection caused a reduction of muscle volume and muscle force, but no change in reflex or passive stiffness. Gene expression results are still in progress. **Conclusions:** Increased stretch reflexes (spasticity) are not accompanied by increased passive stiffness following spinal cord hemisection in rats.

Disclosures: J. Pingel: None. **J. Lorentzen:** None. **J. Wienecke:** None. **J.B. Nielsen:** None.

Nanosymposium

17. Plasticity After Spinal Cord Injury

Location: 143A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 17.08

Topic: D.10. Spinal Cord Injury and Plasticity

Support: DoD Award W81XWH-10-1-0741

NIH: R37 NS30853

Title: Inducing cortico-cortical connectivity to bypass acute cortical impact injury in the rat

Authors: *G. M. VAN ACKER, III[1], D. GUGGENMOS[1], S. BARBAY[1], K. CRABTREE[2], C. DUNHAM[1], R. J. NUDO[1];
[1]Mol. and Integrative Physiol., [2]Neurosurg., Univ. of Kansas Med. Ctr., Kansas City, KS

Abstract: Reconstruction of neural circuitry following acute central nervous system injury remains an ongoing clinical and basic research challenge. Recent studies that applied activity-dependent stimulation (ADS) to promote functional recovery following cortical lesion (Guggenmos et al. 2013) and to entrain cortico-cortical connectivity in the intact brain (Van Acker III et al. 2013) provide encouraging results. The present study expands upon these results by utilizing ADS to potentiate cortico-cortical connectivity immediately status-post controlled cortical impact (CCI) lesion to the caudal forelimb area (CFA) within primary motor cortex. Subsequent to impact in each of 16 ketamine-sedated Long-Evans rats, stimulation was delivered to either somatosensory (S1) forelimb or barrel field (BF) areas using either ADS (n= 8 rats) or random stimulation (RS; n= 8 rats) for 180 minutes. For ADS, neural activity recorded from rostral forelimb area (RFA) was used to trigger stimulus pulses, whereas RS pulses were

delivered independent of biological feedback at approximately 7 Hz (Gaussian distribution). Results suggest that cortico-cortical entrainment immediately status-post CCI injury can be evoked more readily than in the healthy brain (Van Acker III et al. 2013), dependent upon location of cortical stimulation. For example, when ADS was delivered to BF in the injured brain, spikes that occurred within 28 ms following each stimulus pulse (spikes/stimulus) increased significantly from the first 30 minutes of stimulation (5.3 ± 1.5 spikes/stimulus) to the final 30 minutes (12.3 ± 1.4 spikes/stimulus, $p < 0.05$), an increase of 7.0 ± 2.7 spikes/stimulus. In contrast, when stimulation was delivered to BF in the healthy brain, the difference in stimulus-associated spikes between the first and last 30 minutes was not significant (0.7 ± 0.7 spikes/stimulus, $p < 0.05$) (Van Acker III et al. 2013). Additionally, when stimulation was delivered to S1 in the injured brain, there was no significant increase in activity between the first and last 30 minutes (3.0 ± 1.1 and 3.5 ± 2.3 spikes/stimulus, respectively; $p > 0.05$). The difference in potentiation of connectivity between S1 and RFA compared to BF and RFA may be due to differences in preexisting connectivity between the two pathways. There are known direct connections between S1 and RFA, whereas there are minimal-to-no direct connections between BF and RFA. It is possible that immediately status-post CCI, a diaschisis-like effect may have differentially disrupted the neurophysiological integrity of S1-RFA cortico-cortical neurons, resulting in an inability to potentiate functional connectivity.

Disclosures: G.M. Van Acker: None. **D. Guggenmos:** None. **S. Barbay:** None. **K. Crabtree:** None. **C. Dunham:** None. **R.J. Nudo:** None.

Nanosymposium

197. Molecular and Functional Biomarkers of Neurodegeneration

Location: 144A

Time: Sunday, November 16, 2014, 1:00 PM - 2:45 PM

Presentation Number: 197.01

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

Support: CCCRP H_003_2014_WRAIR

Title: Long-term changes in cleavage processing of Alzheimer’s disease related factors APP and Tau following penetrating TBI

Authors: *C. M. CARTAGENA, A. MOUNTNEY, Z. RAMMELKAMP, A. SWIERCZ, D. A. SHEAR, F. C. TORTELLA, K. E. SCHMID;
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Abstract: Traumatic brain injury (TBI) has been established as a risk factor the later development of Alzheimer's disease (AD). Historically both clinical and animal research investigating links between TBI and Alzheimer's disease exclude cases or models of penetrating brain injury, leaving a significant gap in knowledge. In addition, AD studies are most often conducted using familial mutations of amyloid precursor protein (APP) or Tau, mutations that may not represent disease progression in sporadic AD or TBI induced cleavage processing of wild type proteins. Here we investigate in rats the effects of penetrating non-ballistic (NB) brain injury and ballistic-like brain injury (PBBI) on APP and Tau cleavage processing in the acute (first week) and subacute (1 month) periods post-injury. PBBI was induced by probe insertion and the rapid inflation of a balloon injuring 10% of total brain volume while NB injury was induced with probe insertion alone. All changes were compared to sham control. Full length (FL) APP was unaltered with NB but decreased significantly with PBBI at 3 (68%) and7 days (47%). APP beta c-terminal fragments (βCTFs) were increased dramatically at 4 and 24 hr post PBBI (856% and 2942% respectively). Later increases (3 days, 193%; 7 day 729%) were more moderate but persistent, indicating a second wave of APP beta cleavage occurs with PBBI. NB induced a temporally similar but reduced amplitude pattern of increases that was only significant at 7 days (547%). Full length Tau was unaltered at 4 hr but decreased progressively with both NB and PBBI starting at 24 hr and culminating in decreases of 87% and 96% respectively by 7 days post-injury. Importantly, a 22 kDa Tau fragment, known for its involvement in tauopathies, including Alzheimer's disease, increased dramatically following PBBI. Increases also showed a biphasic pattern with more substantial increases at 4hr (1541%) and 24 hr (2367%) and second phase increases at 3 (1541%) and 7 days 2424%). Again NB induced a temporally similar but reduced amplitude pattern of increases which were significant at 4 (544%), and 24 hr (893%) and 7 days (2313%). Pilot studies 1 month post PBBI indicate FL APP is unaltered and βCTFs trend up but are not significantly increased. However, FL Tau remains decreased (68%) and Tau 22 kDa fragment increases persist (468%). These studies show that penetrating brain injury dramatically alters neuropathologic factors related to AD into the subacute period post-injury, indicating that continued research of the relationship between penetrating brain injury and AD is warranted. Ongoing studies will determine if Tau pathology can be detected at more chronic time-points.

Disclosures: C.M. Cartagena: None. **A. Mountney:** None. **Z. Rammelkamp:** None. **A. Swiercz:** None. **D.A. Shear:** None. **F.C. Tortella:** None. **K.E. Schmid:** None.

Nanosymposium

197. Molecular and Functional Biomarkers of Neurodegeneration

Location: 144A

Time: Sunday, November 16, 2014, 1:00 PM - 2:45 PM

Presentation Number: 197.02

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

Support: 5P50AG005681-29

Title: Absolute quantitation of Apolipoprotein E isoforms in human cerebrospinal fluid and brain

Authors: *A. T. BAKER-NIGH[1], K. G. MAWUENYEGA[1], V. OVOD[1], H. ZAKERI[1], T. KASTEN[1], R. J. BATEMAN[1,2,3];
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Abstract: Risk for Alzheimer's Disease (AD) is correlated with Apolipoprotein-E (ApoE) genotype. In humans, the ApoE gene has three major allelic variants that differ by single cystine-arginine replacements. The prevalence for each allele is 78% ApoE3, 15% ApoE4, and 7% ApoE2. While ApoE3 is associated with baseline AD risk and ApoE2 confers decreased risk, ApoE4 is implicated in up to half of sporadic AD cases. The effect is dose-dependent, with ApoE4 heterozygotes 3-fold more likely and homozygotes 12-fold more likely to develop AD. ELISA-based measures of ApoE protein in the CNS do not consistently demonstrate an increase or decrease in ApoE4 levels in carriers. Liquid chromatography with selected reaction monitoring mass spectrometry (LC/SRM) enables the simultaneous detection and quantitation of ApoE isoforms. CSF and Brain Samples: CSF was obtained from 83 individuals (n=42 amyloid positive by CSF Amyloid-β 42/40 ratio or PiB-PET score, and 41 age-matched controls; 44 ApoE33, 35 ApoE34, and 4 ApoE44) and brain from 60 cognitively normal individuals with a range of ApoE genotypes. Estimating relative concentrations: Cross-titrated dilution curves of labeled ApoE3 (increasing) and ApoE4 (decreasing) heavy-arginine (13C6 15N4 L-arginine) media from immortalized murine astrocytes expressing human ApoE were spiked with a consistent volume of mixed unlabeled ApoE3/ApoE4 media to determine the ratio at which common peptides were detected equally. Similar cross-titration experiments were performed using equilibrated labeled media and pooled cerebrospinal fluid (CSF) from ApoE33 or ApoE44 homozygous cases. Consequently, separate and combined standard curves using E33 and E44 CSF were produced. Affinity purification and sample preparation: ApoE was isolated from CSF and brain spiked with media internal standard by affinity purification overnight using Liposorb, a lipophilic absorbant, or by immunoprecipitation using the WUE4 antibody. Samples were then denatured, reduced, and alkylated, followed by protein digestion with trypsin. ApoE isoform-specific peptides LGADMEDVCGR (E3) and LGADMEDVR (E4)and 4 common peptides were selected for analysis. Samples were then analyzed on a NanoAcquity LC coupled to a TSQ Vantage Mass Spectrometer for selected reaction monitoring (SRM) analysis. In this cohort, ApoE4 homozygote CSF demonstrated a decrease of up to 50% in ApoE amount, confirmed by

both specific and common peptide measures. ApoE4 was consistently higher (by ~11%) than ApoE3 in ApoE34 heterozygous cases, and similar in amount to ApoE44 homozygotes. ApoE33 and ApoE34 individuals had similar amounts of total ApoE in their CSF.

Disclosures: **A.T. Baker-Nigh:** None. **K.G. Mawuenyega:** None. **V. Ovod:** None. **H. Zakeri:** None. **T. Kasten:** None. **R.J. Bateman:** 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.; Merck, Research Collaboration, DIAN-TU, NIH U-01-AG042791, DIAN-TU, Alzheimer's Association, Zenith Fellows Award, Alzheimer's Association. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Avid Radiopharmaceuticals, DIAN-TU (donation of imaging supplies). D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents' (e.g., speakers' bureaus); Roche, Invited Speaker. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C2N, Co-Founder/Part Owner. F. Consulting Fees (e.g., advisory boards); Novartis, Sanofi, IMI.

Nanosymposium

197. Molecular and Functional Biomarkers of Neurodegeneration

Location: 144A

Time: Sunday, November 16, 2014, 1:00 PM - 2:45 PM

Presentation Number: 197.03

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

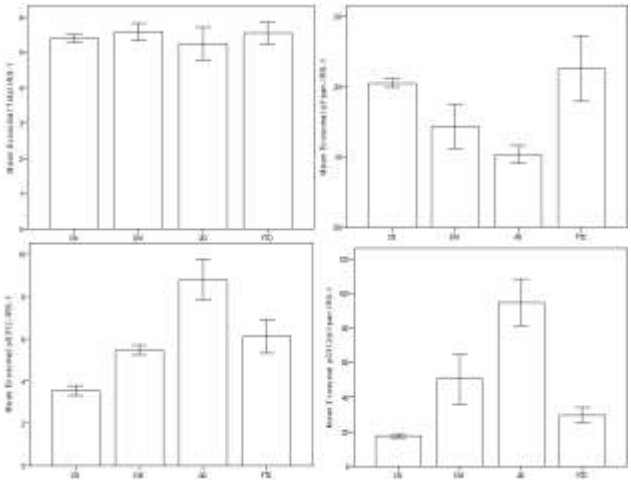
Support: Intramural Research Program of the NIA/NIH

Title: Neural origin plasma exosomes provide novel biomarkers for brain insulin resistance in Alzheimer's disease

Authors: ***D. KAPOGIANNIS**[1,2], A. BOXER[3], E. L. ABNER[5], A. BIRAGYN[1], U. MASHARANI[4], L. FRASSETTO[4], R. C. PETERSEN[6], B. L. MILLER[3], E. J. GOETZL[4]; [1]Natl. Inst. on Aging (NIA/NIH), Baltimore, MD; [2]Neurol., Johns Hopkins Univ., Baltimore, MD; [3]Neurol., [4]Med., UCSF, San Francisco, CA; [5]Univ. of Kentucky, Lexington, KY; [6]Neurol., Mayo Clin., Rochester, MN

Abstract: Introduction: Brain insulin resistance (IR) occurs in Alzheimer's disease (AD), even without peripheral IR. Brain IR is pathogenically important and the target of clinical trials (intranasal insulin, exenatide), but no biomarker of brain IR exists. Recently, high Ser- and low

Tyr-phosphorylated IRS-1 (insulin receptor substrate-1) were proposed as ex-vivo markers of brain IR. Exosomes are endosome-derived vesicles released by various cells (including neurons) and contain proteins reflecting their cellular source. We hypothesized that neural-origin exosomes can be derived from plasma and provide brain IR biomarkers. Methods: Cross-sectional: 48 patients with AD without diabetes, 20 elderly cognitively normal (CN) subjects with diabetes, 16 patients with Frontotemporal Dementia (FTD), and 84 CN controls. Longitudinal: 22 patients with AD with samples 1-10 years before diagnosis. We isolated exosomes from plasma and derived a portion enriched for neural origin by means of expressing NCAM/ L1-CAM. We quantified total IRS-1, p-Ser312-IRS-1, p-panY-IRS-1 (Tyr phosphorylated form) in neural-origin-enriched exosomes and calculated p-Ser312/p-panY ratios. We examined their performance in diagnostic classification with Discriminant Classification (cross-validated by leave-1-out) and Receiver Operating Characteristic (ROC) analyses. Results: AD patients had several-fold higher p-Ser312-IRS-1 and Ser312/p-panY ratios and lower p-panY-IRS-1 than CN, diabetes, and FTD controls (Figure displays means and 95% C.I.; p 98% of AD patients vs. CN controls. The Ser312/p-panY ratio achieved a 0.99 ROC area under the curve. Longitudinally, preclinical and clinical p-Ser312-IRS-1, p-panY-IRS-1, and Ser312/p-panY were indistinguishable; preclinical levels of all three differed vs. controls ($p<0.001$). Conclusions: We propose p-Ser312-IRS-1, p-panY-IRS-1, and Ser312/p-panY from neural-origin plasma exosomes as biomarkers of brain IR in AD. These markers near-perfectly discriminate AD patients vs. CN, diabetes and FTD controls and may predict future AD diagnosis.



Disclosures: D. Kapogiannis: None. **A. Boxer:** None. **E.L. Abner:** None. **A. Biragyn:** None. **U. Masharani:** None. **L. Frassetto:** None. **R.C. Petersen:** None. **B.L. Miller:** None. **E.J. Goetzl:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Nanosomix, Inc..

Nanosymposium

197. Molecular and Functional Biomarkers of Neurodegeneration

Location: 144A

Time: Sunday, November 16, 2014, 1:00 PM - 2:45 PM

Presentation Number: 197.04

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

Support: APVV-0088-10

VEGA 2/0036/11

Brain Centre of SAS

Title: Stress-induced changes in tau proteins and catecholamines in a rat model of Alzheimer´s neurodegeneration

Authors: *R. KVETNANSKY[1], K. LEJAVOVA[2], P. NOVAK[3], A. OPATTOVA[3], K. ONDICOVA[2], L. HORVATHOVA[2], P. VARGOVIC[2], G. MANZ[4], B. MRAVEC[2], P. FILIPCIK[3], M. NOVAK[3];
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Abstract: Stress is one of the factors suspected of promoting neurofibrillary degeneration in Alzheimer´s disease (AD). The aim of this study was to investigate the mutual influences between stress, brain catecholamines (CA) and pathological post-translational modifications of tau protein. The influence of stress on progression of neurodegeneration has been investigated using rats over-expressing human truncated tau protein. Furthermore, corticotropin-releasing hormone (CRH)-knockout mice were utilized to elucidate the role of CRH and corticosteroids in an impact of stress on tau protein modifications. A total of 14 brain areas were analyzed for levels of hyperphosphorylated tau protein, CA, and gene expression of CA-biosynthetic enzyme - tyrosine hydroxylase (TH) in control, singly, and repeatedly stressed animals by immobilization for 2 hours daily (IMO). In both experimental models we found significant hyperphosphorylation of several AD-associated epitopes on tau proteins (pT181, pS202/T205, Ser396/Ser404). Tauopathy induced altered norepinephrine levels in many investigated brain areas and increased expression of TH in brainstem areas, e.g. in the locus coeruleus (A6 area), in A1, A5 areas, etc. The HPA axis has been found to be an important mediator of the hyperphosphorylation response of tau proteins to stress. We have shown that stress induces tau protein phosphorylation throughout the brain, and that absence of CRH delays the onset of hyperphosphorylation. In chronic stress, CRH-producing animals showed an attenuation of the stress response, while

CRH-knockout mice displayed an exaggerated phosphorylation response. This indicates a more complex role of CRH in tau phosphorylation than the current state of the art shows. Our results suggest that stress-induced pathological phosphorylation of tau proteins represents one of the potential mechanisms, which can lead to misfolding of tau proteins and thus to acceleration of neurodegeneration. The results suggest a close interaction between neurofibrillary degeneration and stress, especially repeated or chronic.

Disclosures: **R. Kvetnansky:** None. **K. Lejavova:** None. **P. Novak:** None. **A. Opattova:** None. **K. Ondicova:** None. **L. Horvathova:** None. **P. Vargovic:** None. **G. Manz:** None. **B. Mravec:** None. **P. Filipcik:** None. **M. Novak:** None.

Nanosymposium

197. Molecular and Functional Biomarkers of Neurodegeneration

Location: 144A

Time: Sunday, November 16, 2014, 1:00 PM - 2:45 PM

Presentation Number: 197.05

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

Support: Center for Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB), Göttingen, Germany

Title: Super-resolution microscopy of cerebrospinal fluid biomarkers: a novel tool for diagnostic research in Alzheimer’s Disease

Authors: ***W. I. ZHANG**[1,2], G. ANTONIOS[3], A. RABANO[4], T. PENA-CENTENO[5], T. A. BAYER[3], S. BONN[5], A. SCHNEIDER[6,2,5], S. O. RIZZOLI[1,2];
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Abstract: Beta-amyloid (Abeta) and tau oligomerization play a critical role in Alzheimer’s Disease (AD) pathology. It is currently thought that they should serve as diagnostic markers for AD. Ideally, the perfect diagnostic tool would not only quantify the porportion of Abeta and tau monomers and different aggregate species, but also the sizes of the aggregates. This has not been possible to date since the assemblies are smaller than the diffraction limit of fluorescence

microscopy (~200 nm). To address this, we turned to stimulated-emission depletion (STED) microscopy. This technique has a high enough precision to differentiate dimers/trimers of Aβ from larger aggregates produced in vitro. We immunostained cerebrospinal fluid (CSF) of 37 AD patients and 23 controls and measured the number of Abeta and tau aggregates, as well as their sizes and intensities. In preliminary analysis, a linear discriminant used these parameters to achieve an accuracy of ~97.0% in discriminating AD patients from controls. In contrast, a similar analysis based only on Abeta and tau concentrations, measured via ELISA, resulted in a precision of ~89.7%. In conclusion, we introduce here a precise diagnostic method for AD which may also be used to predict AD at pre-symptomatic stage. In addition, this technique constitutes an unprecedented application of super-resolution microscopy to the medical diagnostic field.

Disclosures: W.I. Zhang: None. **G. Antonios:** None. **A. Rabano:** None. **T. Pena-Centeno:** None. **T.A. Bayer:** None. **S. Bonn:** None. **A. Schneider:** None. **S.O. Rizzoli:** None.

Nanosymposium

197. Molecular and Functional Biomarkers of Neurodegeneration

Location: 144A

Time: Sunday, November 16, 2014, 1:00 PM - 2:45 PM

Presentation Number: 197.06

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

Support: National Science Foundation

NIH: AG017586

NIH: AG032953

NIH: AG038490

NIH: AG043503

NIH: NS044266

NIH: NS053488

Title: Neural correlates of verbal memory and lexical retrieval in Logopenic Variant of Primary Progressive Aphasia

Authors: *K. WIN[1,2], J. PLUTA[3], P. YUSHKEVICH[3], D. WOLK[2], M. GROSSMAN[1,2]; [1]Neurol. Dept, Penn Frontotemporal Degeneration Ctr., Philadelphia, PA; [2]Neurosci. Grad. Group, [3]Penn Image Computing and Sci. Lab., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Motivation: Logopenic variant of primary progressive aphasia (lvPPA) is characterized by poor repetition of phrases and sentences, and limited word-finding. Recently, Flanagan et al. 2014 showed verbal memory deficit in lvPPA but the basis for this is unclear. One possibility is related to discrete atrophy in medial temporal lobe (MTL) substructures, including Cornu Ammonis (CA1), dentate gyrus (DG), subiculum (SUB), entorhinal cortex (ERC), Brodmann areas (BA) 35 and 36. Much research has shown posterior perisylvian atrophy but no MTL atrophy. lvPPA is often associated with Alzheimer's disease (AD), associated with MTL atrophy. Even if there is not overall MTL atrophy in lvPPA, MTL substructures might be differentially affected. T1 MRI of whole-brain grey matter (GM) may be insensitive to detect atrophy of MTL substructures associated with verbal episodic memory (EM) deficit. A second possibility is impaired lexical retrieval may interfere with EM recall. A more reliable assessment of EM may require a recognition testing. Here, we related verbal EM recall and recognition as well as lexical retrieval in lvPPA to MTL substructures using a specialized high resolution T2 MRI sequence, and to GM atrophy using T1 MRI. Methods: Both lvPPA (n=11) and elderly controls (Ctl, n=22) were matched in age, sex, education, and intracranial volume. All subjects underwent T1 MRI as well as a T2 MRI, which maximizes visualization of the dark band separating CA from DG. A multi atlas algorithm was applied to automatically label CA1, DG, SUB, ERC, BA35 and BA36. We used Philadelphia Verbal Learning Test (PVLT) to assess verbal episodic memory and Boston Naming Test (BNT) to measure lexical retrieval. Regression analyses were performed. Results: Compared to Ctl, lvPPA patients performed poorly on forward digit span ($p<0.001$), BNT ($p<0.025$), and PVLT delayed recall ($p<0.008$) although their recognition memory was intact ($p>0.5$). Significant atrophy of MTL substructures was found in right BA35, bilateral CA1 and SUB in lvPPA. Regression analyses showed that only PVLT recall, but not BNT, is associated with left CA1 ($r=0.69$, $p=0.019$). Significant GM atrophy was found in temporal-parietal areas, including middle and inferior temporal, and superior parietal gryi: BNT deficit is related to left superior-parietal atrophy and PVLT to left posterior-inferior temporal atrophy, areas involved in lexical-retrieval and depth of processing effect on encoding of words. Conclusion: We found that specific MTL substructures and temporal-parietal areas are both affected in lvPPA, and regression analyses suggest that impaired lexical retrieval contributes significantly to EM deficits in lvPPA.

Disclosures: K. Win: None. **J. Pluta:** None. **P. Yushkevich:** None. **D. Wolk:** None. **M. Grossman:** None.

Nanosymposium

197. Molecular and Functional Biomarkers of Neurodegeneration

Location: 144A

Time: Sunday, November 16, 2014, 1:00 PM - 2:45 PM

Presentation Number: 197.07

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

Title: Role of entorhinal cortex - hippocampal circuit in Alzheimer's disease mouse model

Authors: *S. A. HUSSAINI, K. DUFF;
Pathology and Cell Biol., Columbia Univ. Med. Ctr., New York, NY

Abstract: Spatial disorientation and confusion in familiar surroundings is an early symptom commonly seen in patients with Alzheimer's disease (AD). The brain regions, entorhinal cortex and hippocampus (known to be important for memory of space), are known to be one of the first regions to undergo pathological changes in Alzheimer's disease. By employing electrophysiological techniques in an AD mouse model we aim to understand the mechanism by which AD affects the neurons involved in spatial memory. Multiple electrodes were implanted in the CA1 region of the hippocampus and medial entorhinal cortex. Animals were allowed to explore an open field environment and subjected to a spatial task. While animals performed the spatial task the activity of neurons in entorhinal cortex and hippocampal was recorded. The hippocampal neurons-place cells- fire at specific locations in an environment representing animal's position. The neurons of entorhinal cortex-grid cells- fire in a grid-like pattern throughout the environment. We analyzed the properties of these neurons in AD mouse and compared with wild-type controls. The firing property of place cell of hippocampus and grid cell of entorhinal cortex was affected in AD mouse model mice. The neuronal properties correlated well with behavioral changes in the AD mice. Additionally, older cohorts performed poorly in behavioral tasks compared to younger cohorts and their neuronal properties were significantly altered. The neuronal properties of entorhinal cortex and hippocampus are significantly affected in an AD mouse model. These properties could potentially serve as a marker for detecting AD pathologies early in the disease.

Disclosures: S.A. Hussaini: None. **K. Duff:** None.

Nanosymposium

198. Neuroinflammation in Alzheimer's Disease

Location: 143A

Time: Sunday, November 16, 2014, 1:00 PM - 3:45 PM

Presentation Number: 198.01

Topic: C.05. Aging

Title: Oxidative stress blocks hippocampal LTP and it is rescue by cGMP, a link to AD

Authors: *L. BUITRAGO-SOTO[1,2], S. ANGULO[2], F. CARDOZO-PELAEZ[3], H. MORENO[2]; [1]Univ. Nacional De Colombia, Bogota, Colombia; [2]Pharmacol., SUNY Downstate Med. Ctr., Brooklyn, NY; [3]Biomed. and Pharmaceut. Sci., Univ. of Montana, Missoula, MT

Abstract: Recent data suggests that cupper levels have been found consistently elevated on MCI and AD and increased Cu levels can serve as predictor of conversion from MCI into AD. In our previous findings, oxidative environments induced by Copper and Ascorbic acid (Cu/Asc) led to the formation and elevation of oxo-8-GTP in a cell-free preparation and in cultured PC12 cells. It has been shown that oxo-8-GTP can act as an inhibitor of the Guanylate Cyclase (GC), significantly reducing cytoplasmatic levels of cGMP. LTP in the hippocampus depends on cGMP, and the inhibition of GC leads to block the induction of LTP in the CA3-CA1 synapsis. The goal of this study is to evaluate oxidative stress induced by Cu/Asc on synaptic plasticity and its molecular targets. Ventral horizontal hippocampal brain slices were obtained from 3 months old male mice. Slices were recorded using aCSF at 34°C. Stimulation electrode was placed in the Schaffer collaterals and evoked field Excitatory postsynaptic potential (fEPSPs) were recorded in the stratum radiatum of CA1. Slices were pre-incubated with Copper 10μM and Ascorbic Acid 1mM for 1h in order to induce the oxidative stress. Control slices were exposed to either normal aCSF or single bath application of copper or ascorbic acid. In a second set of experiments, the slices were pre-incubated with Cu/Asc, then washed-out with normal aCSF for 10 min (Cu/Asc/wo), and subsequently perfused with cGMP 100μM for 10 min. Stable baseline was recorded for 15 min, followed by induction of LTP with a high frequency stimulation (100Hz, 1 s). Slope was taken from the 10-90% of fEPSP, normalized to the baseline and used for the analysis. Synaptic stimulation was recorded for 1h after the induction the LTP and comparisons between groups were made 30 min after the induction. In the first series of the experiments, LTP was induced and maintained up to 1h in control slices. Slope was 2.9, 1.8 and 2 times higher than the baseline in slices exposed to aCSF, Cu and Asc respectively. Potentiation was significantly reduced in the slices exposed to Cu/Asc (1.4). In the second set of experiments, slices in the Cu/Asc/wo protocol had a significant reduction in the potentiation of LTP (1.6). But the potentiation was rescued with the bath application of cGMP (2.4). Thus, a mild oxidative stress challenge with Cu/Asc reduced the potentiation of LTP, which were reversed by the bath addition of cGMP. Our results, combined with previously published work with the Cu/Asc system provide a potential mechanism that can impact biochemical and electrophysiological components related to learning and memory and play a role in these neurological deficits.

Disclosures: **L. Buitrago-Soto:** None. **S. Angulo:** None. **F. Cardozo-Pelaez:** None. **H. Moreno:** None.

Nanosymposium

198. Neuroinflammation in Alzheimer's Disease

Location: 143A

Time: Sunday, November 16, 2014, 1:00 PM - 3:45 PM

Presentation Number: 198.02

Topic: B.11. Glial Mechanisms

Title: Microglial priming with interferon γ is essential for toll-like receptor 4-mediated neurotoxicity

Authors: ***I. PAPAGEORGIOU**[1], A. LEWEN[1], T. REGEN[2], U.-K. HANISCH[2], O. KANN[1]; [1]Med. Fac. Univ. of Heidelberg, Heidelberg, Germany; [2]Inst. of Neuropathology, Med. Ctr. of Georg-August-University, Göttingen, Germany

Abstract: Microglial activation has been associated with the pathogenesis of neurodegenerative diseases such as Parkinson´s disease, multiple sclerosis, Alzheimer´s disease and epilepsy with hippocampal sclerosis. Microglial cells not only remove dead/dysfunctional neurons, but are also proposed to directly induce neuronal death. However, mutual influences between innate (microglia) and adaptive immunity (lymphocytes, natural killer (NK) cells) might blur the individual roles of microglia in in vivo neurotoxicity. AIMS: We aimed to investigate microglial neurotoxicity in organotypic hippocampal slice cultures, a complex in situ approach free of adaptive immunity influences. Microglia are selectively targeted with the toll-like receptor 4 ligand, lipopolysaccharide (LPS), and the neurotoxic impact is investigated in presence or absence of the lymphocytic cytokine interferon γ (IFNγ). METHODS: We characterized microglial activation by a multi-dimensional approach combining high-order morphology, proinflammatory cytokine secretion profile (ELISA) and nitrite production (Griess). Cell numbers were estimated using unbiased, design-based stereology and single cells were reconstructed Neurolucida® for quantification of the soma and branching morphology. The neurotoxic impact was assessed by lactate dehydrogenase activity assay (LDH), neuronal (immuno)histochemistry (Nissl, parvalbumin and neurofilament staining) and extracellular electrophysiological recordings of spontaneous and evoked neuronal activity in the hippocampal CA3 subregion. RESULTS: 1) LPS did not induce neurotoxicity in the absence of interferon γ (IFNγ), despite activation-related morphological changes (i.e. process thickening and somatic enlargement), secretion of proinflammatory cytokines (TNFα, IL6) and production of nitrite.

Both LDH assay and detailed electrophysiology showed that LPS-induced activation was associated with only minor effects on neuronal excitability and short-term plasticity, but no neurodegeneration. 2) Co-incubation of LPS with IFNγ resulted in massive neurodegeneration associated with strong up-regulation of the microglial inducible nitric oxide synthase (iNOS) and high levels of nitrite in the supernatant. 3) LPS/IFNγ toxicity was reversible by pharmacological blockade of iNOS. CONCLUSIONS: We conclude that long-term microglial activation by LPS is not sufficient to drive dysfunction and neuronal death in organotypic hippocampal slice cultures, unless primed with IFNγ. Activation of iNOS and production of nitric oxide is suggested as a critical mediator of LPS/IFNγ toxicity.

Disclosures: I. Papageorgiou: None. **A. Lewen:** None. **T. Regen:** None. **U. Hanisch:** None. **O. Kann:** None.

Nanosymposium

198. Neuroinflammation in Alzheimer's Disease

Location: 143A

Time: Sunday, November 16, 2014, 1:00 PM - 3:45 PM

Presentation Number: 198.03

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

Support: DFG Excellence cluster SyNergy

Title: TREM2 mutations linked to neurodegeneration impair cell surface transport and phagocytosis

Authors: *C. HAASS;
DZNE (German Ctr. for Neurodegenerative Diseases), Munich, Germany

Abstract: Genetic variants in the triggering receptor expressed on myeloid cells 2 (TREM2) have been linked to Nasu-Hakola disease, Alzheimer’s disease (AD), Parkinson's disease, amyotrophic lateral sclerosis, frontotemporal dementia (FTD) and FTD-like syndrome without bone involvement. TREM2 is an innate immune receptor preferentially expressed in microglia and involved in inflammation and phagocytosis. Whether and how TREM2 missense mutations affect TREM2 function is elusive. Here we report that missense mutations associated with FTD and FTD-like syndrome reduce TREM2 maturation, abolish shedding by ADAM proteases and impair phagocytosis. An AD associated mutant TREM2 variant also reduces shedding although to a lower extent. As a consequence of reduced shedding TREM2 is virtually absent in the cerebrospinal fluid (CSF) and plasma of a patient with FTD-like syndrome. Lower levels of

TREM2 were also observed in CSF of AD and FTD patients further supporting that reduced TREM2 function may contribute to the risk for two prominent neurodegenerative disorders.

Disclosures: C. Haass: None.

Nanosymposium

198. Neuroinflammation in Alzheimer's Disease

Location: 143A

Time: Sunday, November 16, 2014, 1:00 PM - 3:45 PM

Presentation Number: 198.04

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

Support: A.P. Giannini Foundation Postdoctoral Fellowship to F.F.R.

NIH R01 MH097268 to P.M.T.

NIH R01 AG040060 to P.M.T.

Title: Alzheimer’s disease risk variant in TREM2 affects ventricular expansion patterns in dementia and normal aging

Authors: *F. F. ROUSSOTTE[1], B. A. GUTMAN[2], D. P. HIBAR[2], S. K. MADSEN[2], P. M. THOMPSON[2];
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Abstract: Introduction We recently reported that elderly carriers of the rs939471 risk allele, a close proxy for a rare variant in TREM2 that triples the lifetime risk of Alzheimer’s disease (AD), annually lost up to 3.3% more brain tissue in the temporal lobes than noncarriers. That study did not control for dementia status. As this TREM2 variant is implicated in multiple neurodegenerative processes, here we hypothesized that the rs939471 risk allele would predict altered trajectories of lateral ventricular expansion, both in dementia and normal aging. Methods We tracked the volume of the lateral ventricles across baseline (N=736), 1-year (N=622), and 2-year (N=479) follow-up scans, in elderly participants from the Alzheimer’s Disease NeuroImaging Initiative. We used general linear models to determine if rs939471 genotype predicted ventricular expansion over a 2-year period, assuming an additive model of allele effects. Results At baseline, the rs939471 risk allele was not significantly associated with total ventricular volume (p=0.323), but it predicted larger volume of the left (p=0.043) but not the right ventricle (p=0.779), after controlling for age, sex, diagnosis, and ApoE status. At both follow-up points, different effects were identified. The same allele was significantly associated

with total ventricular expansion ($p=0.015$ and $p=0.002$ after 1 and 2 years, respectively) and with ventricular enlargement of the right ($p=0.001$ and $p<0.001$) but not the left ventricle ($p=0.129$ and $p=0.102$), after controlling for the same variables. Conclusion This is the first study to show that an AD risk variant in TREM2 is associated with altered trajectories of lateral ventricular enlargement in the elderly. As expected, the risk allele predicts larger ventricular volumes, but the mechanisms and laterality of its effects are unclear. Some have argued that the left hemisphere is affected earlier in AD and may be more susceptible to particular neurodegenerative processes. The largest genome-wide association study for CSF p-tau to date reports a strong association between the TREM2 risk variant and increased CSF p-tau levels, indicating neuronal death due to neurofibrillary tangles. Lateral ventricle expansion typically reflects hippocampal atrophy, and two recent studies suggest that the negative correlation between CSF p-tau levels and hippocampal volumes is left-lateralized. It is thus plausible that the rs939471 risk allele may have delayed effects on the right hemisphere via other, possibly slower neurodegenerative mechanisms, perhaps interfering with the anti-inflammatory and amyloid-β clearance functions of microglial cells expressing the TREM2 receptor.

Disclosures: F.F. Roussotte: None. **B.A. Gutman:** None. **D.P. Hibar:** None. **S.K. Madsen:** None. **P.M. Thompson:** None.

Nanosymposium

198. Neuroinflammation in Alzheimer's Disease

Location: 143A

Time: Sunday, November 16, 2014, 1:00 PM - 3:45 PM

Presentation Number: 198.05

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

Support: NINDS Grant NS084210

The Dana Foundation

Title: Adult human microglia proliferate in culture to high passage and maintain their response to the amyloid-β peptide

Authors: C. GEULA[1], A. REZVANIAN[1], M. PETERSON[1], T. GEFEN[1], S. WEINTRAUB[1], E. BIGIO[1], *M.-M. MESULAM[1], J. EL KHOURY[2], L. GUO[1];
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Abstract: A great deal of what has been learned regarding microglial biology is based on *in vitro* studies the overwhelming majority of which have used cells isolated from the rodent brain. However, higher anatomical and functional complexity of the human brain and species differences in microglial response make imperative the use of human microglia to ascertain that the results obtained are applicable to man. Furthermore, investigation of microglial function in the adult brain, in which many inflammatory and anti-inflammatory microglial responses occur, requires use of adult human microglia. Microglia cultured from embryonic human brains show substantial proliferative capacity. However, while methods for isolation of microglia from adult postmortem human brains exist, they allow use of a limited quantity of microglia isolated and cultured from each case due to low levels of proliferation. We have developed a new technique which allows culturing microglia from postmortem adult human brains to high passage. Gray matter from cortical tissue in frontal poles of 5 cognitively normal aged individuals and 5 patients suffering from Alzheimer's disease (AD) was used. Dissociated cells were cultured in a medium containing microglia growth supplement and granulocyte macrophage colony stimulating factor. Microglia from both normal and AD cortex displayed excellent proliferation to high passage (20 passages, highest attempted). It took 7-10 days for the cells in each passage to reach 70-80% confluence. We did not observe differences in proliferation rate in cultures derived from tissue with different postmortem intervals up to 24 hours. Furthermore, we did not observe differences in rate of growth in cultures from normal brains when compared with brains from AD patients. Cryopreserved microglia displayed similar proliferation when compared with fresh cultures. Cultured cells of various passages displayed immunoreactivity for the specific microglia marker cluster of differentiation (CD)-68, but not for glial fibrillary acidic protein (GFAP), a specific marker of astrocytes. Nearly 100% of the cultured cells endocytosed acetyl low density lipoprotein (Ac-LDL), a ligand for scavenger receptors and a marker of microglia. Cultured microglia from normal and AD cortex produced substantial reactive oxygen species in response to fibrillar amyloid-β (Aβ) peptide, and significantly less in response to oligomeric Aβ. We did not detect differences in response to Aβ in cultures of different passages, nor between cultures from normal and AD brains. In conclusion, adult human microglia proliferate and survive to high passage in culture with maintained function.

Disclosures: **C. Geula:** None. **A. Rezvanian:** None. **M. Peterson:** None. **M. Mesulam:** None. **T. Gefen:** None. **S. Weintraub:** None. **E. Bigio:** None. **J. El Khoury:** None. **L. Guo:** None.

Nanosymposium

198. Neuroinflammation in Alzheimer's Disease

Location: 143A

Time: Sunday, November 16, 2014, 1:00 PM - 3:45 PM

Presentation Number: 198.06

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

Support: NIH AT006816

VA Merit (GMC, SAF)

Mary S Easton Consortium

Title: Targeting alzheimer-related innate immune gene expression with available therapeutics

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Abstract: Background: GWAS and other studies implicate causal roles for innate immune microglial-expressed genes TREM2 and CD33 in Alzheimer Disease (AD) pathogenesis. To date, these studies support protective effects of TREM2 and deleterious effects of CD33. CD33 stimulates SHP tyrosine phosphatase activity opposing the TREM2/ TYROBP tyrosine kinase signaling that leads to CD68 positive phagocytes. CD33 opposes amyloid clearance while TREM2, like anti-abeta immunotherapies, promotes clearance, suggesting therapeutics that increase TREM2/TYROBP and decrease CD33 expression might reduce AD risk.**Methods:** APPsw Tg2576 transgenic mice were treated with immunomodulatory dietary curcumin from 10 to 16 months of age. Amyloid plaque burden and microglial phosphotyrosine (PT) were quantified by ICC while insoluble Abeta and IL-1beta were measured by ELISA. Cortical mRNA for TREM2, TYROBP, CD33, CD68, CD11b, iNOS and Arg-1 were measured by real time qPCR. Curcumin was also used in vitro with primary rodent microglia and cell lines, including human THP-1. **Results:** Dietary low dose curcumin significantly reduced amyloid and increased pro-phagocytic TREM2, TYROBP and CD68 but reduced CD33, microglial marker CD11b and M1-related iNOS and IL-1beta protein. TREM2 co-localized with elevated peri-plaque PT-labeled microglia. TREM2 and TYROBP mRNA levels correlated positively with peri-plaque PT in curcumin treated mice. High dose curcumin significantly reduced M2 marker Arg-1 and failed to lower amyloid or increase the M2 marker TREM2. In vitro, low dose curcumin directly reduced CD33 and increased TREM2 protein levels in rodent and human cell lines. Low dose curcumin increased amyloid phagocytosis and clearance from AD brain sections and increased microglial phagocytosis of beads. **Conclusions:** TREM2 and TYROBP expression are central hubs controlling AD gene expression changes. Our results show that dietary curcumin acts as an immunomodulator by reducing expression of new AD innate immune target gene product CD33 while increasing dementia protective TREM2 expression consistent with increased amyloid clearance and anti-inflammatory activity in vitro and in vivo. These effects are seen with human myeloid lineage cells in vitro and with drug levels that are achievable in

patients with new formulations of curcumin currently in clinical trials at our site. We conclude that curcumin is a strong candidate for controlling innate immune expression related to AD risk.

Disclosures: **G.M. Cole:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); UCLA/VA patent on curcumin formulation Longvida licensed to Verdure Biosciences. **B. Teter:** None. **T. Morihara:** None. **Q. Ma:** None. **X. Zuo:** None. **F. Yang:** None. **S. Frautschy:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); UCLA/VA patent on curcumin formulation Longvida licensed to Verdure Biosciences.

Nanosymposium

198. Neuroinflammation in Alzheimer's Disease

Location: 143A

Time: Sunday, November 16, 2014, 1:00 PM - 3:45 PM

Presentation Number: 198.07

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

Support: Cure Alzheimer's Fund

NIH Grant F32-NS083187-01A1

NMSS JF 2144A2/1

Title: Altered microglial response to Abeta plaques in APPPS1-21 mice heterozygous for TREM2

Authors: ***J. D. ULRICH**[1], M. FINN[1], Y. WANG[2], A. SHEN[1], T. E. MAHAN[1], H. JIANG[1], F. R. STEWART[1], L. PICCIO[1], M. COLONNA[2], D. M. HOLTZMAN[1];
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Abstract: Recent genome-wide association studies linked variants in TREM2 to a strong increase in the odds of developing Alzheimer’s disease, however the mechanism by which TREM2 influences the susceptibility to Alzheimer’s disease is currently unknown. Within the brain, TREM2 is expressed by microglia and is thought to regulate microglial phagocytic and inflammatory responses to pathological insults. Since a single allele of variant TREM2, that is hypothesized to be detrimental to TREM2 function, conferred an increased risk of developing Alzheimer’s disease, we tested whether loss of one functional trem2 allele would affect Aβ plaque deposition or the microglial response to Aβ pathology in APPPS1-21 mice. We observed

no significant difference in Aβ deposition in 3-month old or 7-month old APPPS1-21 mice expressing one or two copies of trem2. However, 3-month old mice with one copy of trem2 exhibited a marked decrease in the size and number of microglia associated with Aβ plaques. While there was no statistically significant differences in cytokine levels or markers of microglial activation in 3- or 7-month old animals, there were trends towards decreased expression of NOS2, C1qa, and IL1a in 3-month old TREM2+/- vs. TREM2+/+ mice. Therefore, we found that loss of a single copy of trem2 had no effect on Aβ deposition, but altered the morphological phenotype of plaque-associated microglia. These data suggest that TREM2 regulates the microglial response to Aβ deposition but does not affect Aβ plaque burden.

Disclosures: **J.D. Ulrich:** None. **M. Finn:** None. **Y. Wang:** None. **T.E. Mahan:** None. **H. Jiang:** None. **F.R. Stewart:** None. **L. Piccio:** None. **M. Colonna:** None. **D.M. Holtzman:** None. **A. Shen:** None.

Nanosymposium

198. Neuroinflammation in Alzheimer's Disease

Location: 143A

Time: Sunday, November 16, 2014, 1:00 PM - 3:45 PM

Presentation Number: 198.08

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

Support: NIH RO1AG030209

NIH R21AG033914

Alzheimer's Association

American Federation for Aging Research

Swedish Research Council

NIH NRSA F31AG039195

NIH NRSA 1F31NS074712

Title: Microglial deletion of the PGE2 receptor EP2 restores immune and trophic responses and rescues cognitive function in Alzheimer’s disease models

Authors: ***K. I. ANDREASSON**[1], J. JOHANSSON[1], N. WOODLING[1], X. LIANG[1], Q. WANG[1], H. BROWN[1], M. PANCHAL[1], T. LOUI[1], A. TRUEBA-SAIZ[2], S. PRADHAN[1];

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Abstract: Microglia, the innate immune cells of the central nervous system, perform critical inflammatory and non-inflammatory functions to maintain local homeostasis and normal neural function. However in Alzheimer's disease (AD), these beneficial functions become progressively impaired, contributing to dysregulated and toxic inflammatory responses, synaptic and neuronal loss, and ultimately cognitive impairment. The inflammatory cyclooxygenase-PGE_2 pathway has been implicated in pre-clinical AD development, both in human epidemiology and in transgenic murine models of AD. In our studies using in vitro and in vivo conditional knockout approaches, we have determined that in mouse models of AD, cell-specific deletion of the microglial PGE_2 EP2 receptor restores microglial chemotaxis and Aß-clearance activity, promotes resolution of toxic inflammatory responses, and increases expression of cytoprotective insulin-like growth factor 1 and Akt signaling. We also find that cell-specific ablation of microglial EP2 prevents onset of hippocampal-dependent memory deficits in AD model mice. In human cerebral cortex, microglial EP2 receptor levels increase as subjects progress from normal aging to mild cognitive impairment to AD. In its overall regulation of distinct microglial functions, our findings indicate that EP2 signaling is a general suppressor of multiple beneficial processes that falter in microglia in the development of AD pathology. Inhibition of inflammatory EP2 signaling may represent a novel approach to restore healthy microglial function that can prevent and delay the development of AD.

Disclosures: K.I. Andreasson: None. **J. Johansson:** None. **N. Woodling:** None. **X. Liang:** None. **Q. Wang:** None. **H. Brown:** None. **M. Panchal:** None. **T. Loui:** None. **S. Pradhan:** None. **A. Trueba-Saiz:** None.

Nanosymposium

198. Neuroinflammation in Alzheimer's Disease

Location: 143A

Time: Sunday, November 16, 2014, 1:00 PM - 3:45 PM

Presentation Number: 198.09

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

Support: NIH grant NS079637

NIH grant P20GM103486

NCRR 5P20RR020171

Title: Determining the role of an M2a phenotype on microglial activity and amyloid deposition using BV2 microglial cells and APP/PS1 transgenic mice

Authors: *C. H. LATTA[1], T. L. SUDDUTH[1], E. M. WEEKMAN[2,1], H. M. BROTHERS[1], F. GONZALEZ OREGON[1], K. J. BRAUN[1], D. M. WILCOCK[2,1];
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Abstract: Microglia are considered to be the resident macrophages of the brain. In their resting state, microglia extend ramified process that probe the brain parenchyma for pathogenic activity and damage. In response to detrimental stimuli, a course of inflammation governs a polarized spectrum of microglial phenotypes based on peripheral macrophage profiles; M1, M2a, M2b and M2c. Classically activated microglia, M1, express pro-inflammatory cytokines as well as oxygen and nitrogen radicals. This phase is frequently termed "a double edged sword"; a toxic environment eradicates any pathogenic activity, yet is destructive to nervous tissue. The transition to an alternative state, M2, establishes a habitable environment permitting repair and regeneration. An M2a phenotype increases the production of anti-inflammatory cytokines and extracellular matrix remodeling proteins consequently dampening the pro-inflammatory response and aiding wound healing. This study aimed to determine the effect of an M2a phenotype on Alzheimer's disease (AD) pathological progression with in vitro and in vivo models. Our approach is to define the inflammatory state of microglia in response to external stimuli such as cytokine application. IL-4 is a strong M2a polarizing cytokine in macrophages but is not secreted by microglia. To initially characterize an M2a phenotypic change in microglia, we used BV2 microglial cells to study the temporal progression of microglial responses to IL-4. The BV2 cells were incubated in serum-free DMEM/F12 media containing murine IL-4. To assess the effect of the released factors on the hallmark pathologies of AD, media was extracted after 8 hours of incubation ,the optimal M2a state, and transferred to CHO APP cells (secreting β-amyloid) and Hek WT Tau and P301L cells (expressing human wild type tau and a tau variant respectively). Additionally, we intracranially injected an adeno-associated viral (AAV) vector to express IL-4 in the frontal cortex and hippocampus of APP/PS1 transgenic mice (which overexpress β-amyloid) at 3 months of age and we evaluated the mice 6 weeks post-injection. Quantitative real-time PCR was used to assess biomarker expression of microglial phenotypes in both the animal tissue and the BV2 cells. Protein analysis was performed on the brain tissue and cell cultures to quantify β-amyloid and tau depositions. Histological staining permitted quantification of microglial activity. Both models showed enhanced M2a phenotypic expression and IL-4 treatment revealed a trend of decreased β-amyloid in the animal models. In summary, this study offers insight into the therapeutic potential of modulating microglial immune response in AD.

Disclosures: C.H. Latta: None. **T.L. Sudduth:** None. **E.M. Weekman:** None. **H.M. Brothers:** None. **F. Gonzalez Oregon:** None. **K.J. Braun:** None. **D.M. Wilcock:** None.

Nanosymposium

198. Neuroinflammation in Alzheimer's Disease

Location: 143A

Time: Sunday, November 16, 2014, 1:00 PM - 3:45 PM

Presentation Number: 198.10

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

Support: NIA Grant AG023012

NINDS Grant NS047804

NINDS Grant NS087298

DOD Grant W81XWH12-1-0629

Bright Focus Foundation A2013252F

NRSA T32 NS067431

A generous donation from Chet and Jane Scholtz

Title: Trem2 expression is upregulated in myeloid cells surrounding plaques in Alzheimer's disease and Trem2 deficiency modulates pathology

Authors: *T. R. JAY[1,2,5], C. M. MILLER[2], L. C. GRAHAM[5], S. BEMILLER[2], C. KARLO[1], G. XU[2], S. STAUGAITIS[2], L. BEKRIS[3], J. LEVERENZ[4], G. HOWELL[5], R. RANSOHOFF[2], G. LANDRETH[1], B. T. LAMB[2];
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Abstract: Alzheimer's disease (AD) is characterized by extracellular accumulation of beta amyloid (Aβ), intraneuronal accumulation of microtubule associated protein tau (MAPT), and by aberrant neuroinflammation. The integral role of inflammation in AD pathogenesis was highlighted by recent studies which showed that mutations in Trem2, an important modulator of myeloid cell activation, confer high risk for developing AD. Using RNA and protein analyses, we found that Trem2 expression is upregulated in three mouse models of Alzheimer's disease and in human AD tissue. Immunohistochemistry and Trem2lacZ/lacZ knock-in mice revealed that the upregulation of Trem2 is localized within Aβ plaque-associated myeloid cells. Our data also suggest that loss of Trem2 in these AD mouse models modulates Aβ and MAPT-related

pathologies. These data explore a possible pathogenic mechanism underlying Trem2 mutations, which will be important to more fully understand the role of inflammation in AD.

Disclosures: **T.R. Jay:** None. **C.M. Miller:** None. **S. Bemiller:** None. **G. Xu:** None. **C. Karlo:** None. **L. Bekris:** None. **S. Staugaitis:** None. **J. Leverenz:** None. **G. Landreth:** None. **G. Howell:** None. **R. Ransohoff:** None. **B.T. Lamb:** None. **L.C. Graham:** None.

Nanosymposium

198. Neuroinflammation in Alzheimer's Disease

Location: 143A

Time: Sunday, November 16, 2014, 1:00 PM - 3:45 PM

Presentation Number: 198.11

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

Support: Jane and Lee Seidman Fund

NIA AG023012 to B.T.L.

NINDS NS047804 to B.T.L.

NINDS NS087298 to B.T.L. and R.M.R.

DOD W81XWH12-1-0629 to B.T.L.

BrightFocus Foundation A2013252F to C.M.M.

NRSA T32 NS067431 to T.R.J.

Title: Trem2 is predominantly expressed by infiltrating monocytes in multiple mouse models of Alzheimer’s disease

Authors: *C. M. MILLER[1], T. R. JAY[1,2], L. C. GRAHAM[3], A. COTLEUR[1], G. LANDRETH[2], G. HOWELL[3], R. M. RANSOHOFF[1], B. T. LAMB[1,2];
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Abstract: Mutations in triggering receptor expressed on myeloid cells 2 (Trem2) were recently shown to confer high risk for developing Alzheimer’s disease (AD) as well as other neurodegenerative diseases. It was anticipated that Trem2 would be expressed predominantly on microglia, the brain-resident myeloid cell population. To test this hypothesis and determine

which myeloid cells in the brain express TREM2, flow cytometry was performed in three different mouse models of AD followed by RNA sequencing. Interestingly, there was an age-dependent increase in the percentage of TREM2-expressing CD11b+/CD45^{hi} peripheral monocytes versus CD11b+/CD45^{lo} microglia. Additional flow and immunohistochemical labeling with F4/80 and Ly6C further established macrophage lineage and monocytic cell identity, respectively. These data suggest that Trem2 is predominantly expressed on macrophages derived from peripheral monocytes in mouse models of AD. Taken together, these results indicate that examining peripheral monocytes will be integral to understanding how Trem2 mutations contribute to the pathogenesis of AD and further delineate the divergent roles of microglia and monocytes.

Disclosures: C.M. Miller: None. **T.R. Jay:** None. **L.C. Graham:** None. **A. Cotleur:** None. **G. Landreth:** None. **G. Howell:** None. **R.M. Ransohoff:** None. **B.T. Lamb:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.01

Topic: C.03. Parkinson’s Disease

Support: Michael J. Fox Foundation

Title: Cerium oxide nanoparticles as a disease-modifying therapy for Parkinson's disease

Authors: A. S. FREY[1], B. LOCKLER[1], M. J. BILLINGS[1], K. S. HOCKEY[1], C. A. SHOLAR[1], *B. A. RZIGALINSKI[2];
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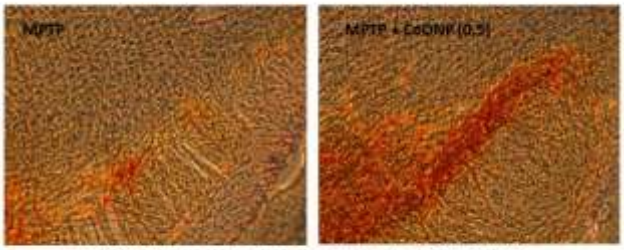
Abstract: Cerium oxide nanoparticles (CeONP) are highly efficient mitochondrial protectants and regenerative free radical scavengers. Our prior work in the MPTP-mouse model of Parkinson's disease, found that administration of CeONP prior to MPTP challenge could completely protect mice from dopaminergic loss. In the present work, we tested the hypothesis that CeONP may be a disease-modifying therapy for Parkinson’s disease, when delivered after development of the disease. C57Bl/6 mice were treated with 20 mg/kg MPTP given in 4 injections spaced 2 hrs apart. CeONP (0.05-5.0 micrograms/g) was delivered intravenously in a single dose, 12 hrs after the last MPTP injection. Seven days later, mice were euthanized and dopamine content in the striatum was measured. Dopaminergic neurons in the substantia nigra

were stained and sterotaxically counted. Lipid peroxidation levels in the brain were also measured. We found that: • CeONP increased the levels of TH+ neurons in the substantia nigra, when delivered alone (No MPTP) • CeONP preserved striatal dopamine by approximately 50%, when delivered after development of the disease. • CeONP preserved dopaminergic neurons in the substantia nigra to 84-87% of controls when delivered after development of the disease • CeONP decreased basal levels of lipid peroxidation in the cortex These results suggest that CeONP may halt or slow disease progression. Further, the ability of CeONP to promote growth of neurons in the substantia nigra suggests the potential to reverse



CeONP preserves dopaminergic neurons in the striatum of MPTP-treated mice when delivered 12 hrs after MPTP challenge.

PD.

Disclosures: **A.S. Frey:** None. **B. Lockler:** None. **M.J. Billings:** None. **K.S. Hockey:** None. **C.A. Sholar:** None. **B.A. Rzigalinski:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.02

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Michael J. Fox Foundation

NIH Grant P01 ES016738

NIH Grant P01 HD29587

NIH Grant P30 NS076411

Title: Transnitrosylation from DJ-1 to PTEN attenuates neuronal cell death in Parkinson's disease models

Authors: *T. NAKAMURA[1], M.-S. CHOI[1], S.-J. CHO[2], E. A. HOLLAND[1], J. QU[1], G. A. PETSKO[3], R. C. LIDDINGTON[2], S. A. LIPTON[1];
[1]Neurosci. and Aging Res. Ctr., [2]Program on Infectious Dis., Sanford-Burnham Med. Res. Inst., LA JOLLA, CA; [3]Weill Cornell Med. Col., New York, NY

Abstract: Emerging evidence suggests that oxidative/nitrosative stress, as occurs during aging, contributes to the pathogenesis of Parkinson's disease (PD). In contrast, detoxification of reactive oxygen and nitrogen species (ROS/RNS), can protect neurons. DJ-1 has been identified as one of several recessively-inherited genes whose mutation can cause familial PD, and inactivation of DJ-1 renders neurons more susceptible to oxidative stress and cell death. DJ-1 is also known to regulate phosphatase and tensin homolog (PTEN) activity, which plays a critical role in neuronal cell death in response to various insults. However, mechanistic details delineating how DJ-1 regulates PTEN activity remain unknown. Here, we report that PTEN phosphatase activity is inhibited via a transnitrosylation reaction, i.e., transfer of an NO group from the cysteine residue of one protein to another. Specifically, we show that DJ-1 is S-nitrosylated (forming SNO-DJ-1); subsequently, the NO group is transferred from DJ-1 to PTEN by transnitrosylation. Moreover, we detect S-nitrosylated (SNO)-PTEN in human brains of sporadic PD. Using X-ray crystallography and site-directed mutagenesis, we find that Cys106 is the site of S-nitrosylation on DJ-1 and mutation of this site inhibits transnitrosylation to PTEN. Importantly, S-nitrosylation of PTEN decreases its phosphatase activity, thus promoting cell survival. These findings provide mechanistic insight into the neuroprotective role of SNO-DJ-1 by elucidating how DJ-1 detoxifies NO via transnitrosylation to PTEN. Dysfunctional DJ-1, which lacks this transnitrosylation activity due to mutation or prior oxidation (e.g. sulfonation) of the critical cysteine thiol, could thus contribute to neurodegenerative disorders like PD.

Disclosures: T. Nakamura: None. **M. Choi:** None. **S. Cho:** None. **E.A. Holland:** None. **J. Qu:** None. **G.A. Petsko:** None. **R.C. Liddington:** None. **S.A. Lipton:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.03

Topic: C.03. Parkinson's Disease

Support: MJ Fox Foundation

Title: Fractalkine over expression suppresses α-synuclein mediated neurodegeneration

Authors: *K. R. NASH[1], D. MORGAN[2], P. C. BICKFORD[2];
[1]Mol. Pharmacol. and Physiol., Univ. of South Florida, Tampa, FL; [2]USF, Tampa, FL

Abstract: In Parkinson's disease (PD) α-synuclein activates microglia and this activation has been suggested as one of the mechanisms of neurodegeneration. There are several signals produced by neurons that have an anti-inflammatory action on microglia, including CX3CL1 (fractalkine). We have previously shown that a soluble form of CX3CL1 is required to reduce neuron loss in MPTP treated mice and that fractalkine agonism can reduce neuron loss in a 6-hydroxydopamine lesion model. Here we show that fractalkine can reduce α-synuclein mediated neurodegeneration in rats. Rats that received fractalkine showed abrogated loss of tyrosine hydroxylase and Neu-N staining. This was replicated in animals where we expressed fractalkine from astrocytes with the GFAP promoter. Interestingly, we did not observe a rescue of neuron loss with the membrane associated form of fractalkine. Further, we did not observe a reduction in MHCII expression suggesting that soluble fractalkine is likely altering the microglial state to a more neuroprotective one rather than reducing antigen presentation. We report that fractalkine receptor agonism with the soluble FKN can rescue neuron loss in the recombinant adeno-associated virus (rAAV) mediated α-synuclein model of PD and warrants further investigation as a therapeutic target.

Disclosures: K.R. Nash: None. **D. Morgan:** None. **P.C. Bickford:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.04

Topic: C.03. Parkinson's Disease

Support: NIH RO1 Grant NS70898

Title: MicroRNA-7 targets RelA to improve cellular bioenergetics and protect against MPP+-induced cell death

Authors: A. DATTA CHAUDHURI[1], S. KABARIA[2], D.-C. CHOI[1], M. MOURADIAN[2], *E. JUNN[3];

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Abstract: Mitochondrial dysfunction and aberrant cellular bioenergetics are hallmarks of Parkinson's disease (PD). These pathological features of the disease can be mimicked in vitro by treating dopaminergic cells with the mitochondrial complex I inhibitor 1-methyl-4-phenylpyridinium (MPP+). In this paradigm, as oxidative phosphorylation is blocked, cells become dependent on glycolysis to meet their energy demands. Increasing the rate of glycolysis can, therefore, rescue cells from MPP+-induced death. MicroRNA-7 (miR-7) is a small, non-coding RNA that is protective in PD models by reducing α-synuclein expression. In the present study, we show that miR-7 also protects against MPP+-induced cytotoxicity. Overexpression of miR-7 in SH-SY5Y cells and differentiated ReNCell VM cells (mesencephalic neural progenitor cell line) prevented cell death and loss of neurites, respectively, induced by MPP+. This protective effect of miR-7 was mediated through down-regulating its target mRNA RelA. Knocking down RelA with siRNA had a similar protective effect. Considering that RelA knockdown increases the rate of glycolysis, we sought to examine whether the mechanism of cytoprotection provided by miR-7 also involves an increase in glycolytic rate. Treatment of SH-SY5Y cells with MPP+ resulted in an expected decrease in ATP production attributed to loss of complex I activity. On other hand, overexpression of miR-7 or knockdown of RelA augmented the rate of glycolysis as evidenced by an in increase in ATP production, glucose consumption and lactate production. Furthermore, both miR-7 and siRelA failed to protect against MPP+-induced cell death when cells were cultured in a low glucose (1 g/L) media instead of regular media containing 4.5 g/L glucose. The latter finding indicates that availability of the glycolytic substrate is required for the observed protective effect. We can, therefore, conclude that miR-7, through down-regulating RelA, promotes glycolysis in order to sustain energy production in the absence of complex I activity and protects against the cytotoxic effect of MPP+.

Disclosures: A. Datta Chaudhuri: None. **E. Junn:** None. **D. Choi:** None. **S. Kabaria:** None. **M. Mouradian:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.05

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Deutsche Forschungsgemeinschaft

Title: Influence of the Ca2+-independent phospholipase A2 (iPLA2) and docosahexaenoic acid on the electron-transport-chain dependent ROS generation in brain mitochondria

Authors: *G. REISER, C. NORDMANN, P. SCHÖNFELD;
Otto-von-Guericke Univ., D39120 Magdeburg, Germany

Abstract: Drug-based prevention of reactive oxygen species (ROS) generation is important, because oxidative stress is a factor for the pathogenesis of many diseases, like neurodegeneration in Alzheimers disease and many others. The Ca2+-independent phospholipase A2 (iPLA2) liberates free fatty acids (FFA) by hydrolyzing the sn-2 ester bond of membrane glycerophospholipids. iPLA2 has been found in various mammalian mitochondria. According to a current concept, the activity of the inner mitochondrial membrane-associated iPLA2 removes oxidatively damaged fatty acids for lipid remodeling and repair. Mitochondria are main ROS producers in cells. Thus, the question arises, whether iPLA2 has antioxidative defense, and attenuates the formation of electron transport chain (ETC)-associated superoxide (O2•-), and thereby reduces oxidative stress. Mild-uncoupling is a suggested mechanism. We examined the influence of iPLA2 on ETC-associated ROS generation in rat brain mitochondria (RBM). First, we used docosahexanoic acid (DHA). DHA is a major reaction product of iPLA2, and was used to adjust mild-uncoupling in succinate-oxidizing RBM and, to impair reversed electron transport (RET) in ETC. We find that low DHA concentrations diminish RET-dependent ROS generation by mild-uncoupling. This was largely due to adenine nucleotide translocase. However, when mitochondria oxidize glutamate plus malate and, thereby support the forward electron transport (FET), DHA enhanced mitochondrial ROS generation. In addition, to reduce the endogenous mitochondrial pool of FFA, mitochondria were treated with the specific iPLA2-inhibitor bromoenol lactone (BEL). BEL-treated (succinate-oxidizing) mitochondria show enhanced ROS generation, but are slightly depolarized in comparison to untreated control. This contradicts the view that iPLA2 inhibition abolishes mild uncoupling and, consequently, enhances the mitochondrial membrane potential. Our novel hypothesis is that we explain mechanistically the increase of the ROS release by BEL-treated RBM with a diminished content of reduced glutathione. Thus, we disprove the concept that iPLA2 attenuates oxidative stress in brain mitochondria. Further work will elucidate mechanisms of pathogenesis of neurodegenerative disorders based on the dysregulated iPLA2. We anlyse this question in a genetic mouse model of INAD: Strokin, M., Seburn, K.L., Cox, G.A., Martens, K.A., Reiser, G.,. Severe disturbance in the Ca2+ signaling in astrocytes from mouse models of human infantile neuroaxonal dystrophy (INAD)with mutated Pla2g6. Hum. Mol. Genet. 21, 2012, 2807-2814

Disclosures: **G. Reiser:** None. **P. Schönfeld:** None. **C. Nordmann:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.06

Topic: C.03. Parkinson’s Disease

Support: Midwestern University Intramural Funds

Title: Exploring the effects of nicotine on NADH dehydrogenase activity and mitochondrial morphology in a *Drosophila* model of Parkinson's disease

Authors: *L. M. BUHLMAN[1], M. ODUMOSU[1], G. B. CALL[2];
[1]Biomed. Sci., [2]Arizona Col. of Med., Midwestern Univ., Glendale, AZ

Abstract: NADH dehydrogenase (mitochondrial respiratory chain complex 1) deficiency is implicated in both sporadic and genetic forms of Parkinson's disease (PD). Mitochondrial toxins that inhibit electron transport from complex 1 are widely used to created sporadic models of PD, while patients with autosomal recessive-juvenile parkinsonism caused by mutations in PARK2 exhibit decreased complex 1 activity as measured in cultured fibroblasts. Nicotine pretreatment has been shown to be beneficial in sporadic PD models, and it can increase median lifespan and improve motor and olfactory deficits in Parkin loss-of-function Drosophila. The mechanism by which nicotine offers protection against the mutant phenotype in Drosophila is unclear. Because nicotine has been shown to bind to complex 1 and affect its activity, we hypothesize that nicotine ameliorates the Parkin loss-of-function phenotype by restoring normal function of complex 1. To this end, we performed in-gel activity assays on mitochondrial fractions from heads of adult Drosophila raised on nicotine and found that nicotine has different effects on complex 1 in mutants compared to control flies. Because Parkin-loss-of-function affects mitochondrial fission events required for disposal of poorly-functioning mitochondria (mitophagy), we explored the possibility that our mutant flies would exhibit aberrant mitochondrial morphology and turnover, which could be particularly detrimental for cells containing mitochondria with complex 1 deficits. Thus, we measured mitochondrial morphology and total mass per cell in TH-expressing neurons of adult Parkin loss-of-function Drosophila brains. Our results shed light on whether aberrant mitochondrial morphology and complex I function play a role in the manifestation of parkinsonism and whether nicotine exerts protective effects by restoring mitochondrial morphology and function.

Disclosures: L.M. Buhlman: None. **M. Odumosu:** None. **G.B. Call:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.07

Topic: C.03. Parkinson's Disease

Support: NIH Grant R01AG038961

NIH Grant R01EB009041

Title: Neuroprotective effects induced by focused ultrasound-facilitated AAV-GDNF delivery in a Parkinson's-disease mouse model

Authors: *S. WANG[1], O. OLUMOLADE[2], V. JACKSON-LEWIS[3], J. BLESA[3], T. SUN[2], G. SAMIOTAKI[2], S. PRZEDBORSKI[3], E. KONOFAGOU[2];
[2]Dept. of Biomed. Engin., [3]Dept. of Pathology and Cell Biol., [1]Columbia Univ., New York, NY

Abstract: The pathology of Parkinson's Disease (PD) is characterized by the relatively selective death of nigro-striatal dopaminergic neurons. Utilizing recombinant adeno-associated virus (rAAV), therapeutic genes can be delivered to the brain for long-lasting treatments. However, the existence of the blood-brain barrier (BBB) prevents efficient delivery of the systemically administered viral vectors. Transcranial focused Ultrasound (FUS) in combination with microbubbles (MB) has been shown capable of inducing reversible blood-brain barrier (BBB) opening. In this study, we investigate the neuroprotective effects of non-invasively delivered rAAV-GDNF vectors after FUS induced BBB opening in a PD mouse model. Animals were divided into four groups (n = 4-6 per group): control, FUS only, rAAV injection only, and rAAV+FUS. For the FUS only and AAV+FUS groups, both striatum (Str) and substantia nigra (SN) were sonicated unilaterally at 1.5 MHz. For the rAAV+FUS group, immediately before sonication, a 100 μl mixture of rAAV1-CAG-hGDNF-GFP vectors (8.5×1011 GC/animal) and in-house polydispersed microbubbles (~2.5×107 #/animal) were administered IV. After 4-week survival, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was administered at 30 μg/kg IP over five consecutive days. Twenty-seven days after the last MPTP injection, the mice were sacrificed and their brains were prepared for tyrosine hydroxylase (TH) staining for subsequent TH-positive neuronal counting in SN. The optical density (OD) and the integrated OD (IOD) were quantified in MATLAB (Mathworks). The ratio of ipsilateral (FUS treated side) to contralateral side with TH-positive neurons in the AAV+FUS group was significantly higher ($p=0.03$) compared to all other groups. The OD and IOD of TH levels in the caudate-putamen was only statistical significant in the AAV+FUS group ($p=0.0059$ and $p=0.0063$, respectively)

compared to all other groups. The IOD of the TH level in the caudate-putamen further confirmed the neuroprotective effects of the non-invasively delivered rAAV-GDNF vectors FUS in combination with MB provide a non-invasive and targeted approach for gene delivery to specific brain targets. This study, for the first time, demonstrated neuroprotective effects of non-invasively delivered rAAV1-GDNF using transcranial FUS in a PD animal model.

Disclosures: **S. Wang:** None. **O. Olumolade:** None. **V. Jackson-Lewis:** None. **J. Blesa:** None. **T. Sun:** None. **G. Samiotaki:** None. **S. Przedborski:** None. **E. Konofagou:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.08

Topic: C.03. Parkinson’s Disease

Support: American Parkinson Disease Association

Parkinson's Association of Alabama

Title: 14-3-3s regulate alpha-synuclein release and toxicity

Authors: ***T. A. YACOUBIAN**, B. WANG;
Dept Neurol, Univ. Alabama Birmingham, BIRMINGHAM, AL

Abstract: Alpha-synuclein (αsyn) plays a critical role in Parkinson’s disease. Recent research suggests a prion-like mode for syn toxicity: αsyn is released as aggregated species that cause further aggregation and toxicity in neighboring cells. We have been investigating the role of the 14-3-3 proteins in regulating alpha-synuclein release and paracrine toxicity. 14-3-3s are chaperone-like proteins that reduce protein aggregation, regulate protein secretion, and promote cell survival. To examine the effect of 14-3-3s on αsyn release, we created a doxycycline (doxy)-inducible αsyn neuroblastoma line (isyn) that upon doxy treatment releases αsyn into conditioned media (CM) that is toxic to separately-cultured primary neurons. We observed that 14-3-3θ overexpression (OE) in isyn cells increased the total amount of αsyn released into CM by 3-fold compared to control isyn cells upon doxy induction for 96 hours. This increase in αsyn release with 14-3-3θ OE was noted as early as 48 hours and maintained at all time points examined up to 7 days. Fractionation of CM into exosomal and non-exosomal fractions using high ultracentrifugal spins revealed that αsyn levels were increased in exosomes but not in the non-

exosomal fraction with 14-3-3θ OE in isyn cells. Despite the increase in αsyn release, we observed a complete elimination of the toxicity of αsyn-enriched CM on separately cultured differentiated SH-SY5Y cells or primary hippocampal neurons. Conversely, 14-3-3 inhibition with the pan-14-3-3 inhibitor difopein caused a 40% decrease in αsyn release into the CM compared to control. This reduction in release was observed primarily in the exosomal fraction but was also seen in the non-exosomal fraction. Difopein in the isyn cells increased the toxicity of αsyn-enriched CM on target cells. To test whether 14-3-3θ OE affects the amount of monomeric vs. oligomeric αsyn released by cells, we used a bioluminescent protein-fragment complementation assay in which luciferase signal is generated when αsyn fused to a non-bioluminscent amino terminal luciferase fragment (S1) interacts with αsyn fused to a carboxy-terminal luciferase fragment (S2). The amount of luciferase signal was significantly reduced with 14-3-3θ OE in H4 cells transfected with S1-syn and S2-syn. Conversely, difopein increased luciferase signal compared to control. Based on these findings, we conclude that 14-3-3θ can regulate the release and toxicity of αsyn and may serve as a target for therapeutic intervention in Parkinson's disease.

Disclosures: **T.A. Yacoubian:** None. **B. Wang:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.09

Topic: C.03. Parkinson's Disease

Support: Dartmouth SYNERGY K12 Program-Early Investigators

Title: Prodromal anti-inflammatory intervention blocks leukocyte infiltration and the progression of Parkinsonism in rotenone treated mice

Authors: *M. C. HAVRDA;
Geisel Sch. of Med. at Dartmouth, Lebanon, NH

Abstract: In the Farming and Movement Evaluation Study, led by the National Institute of Environmental Health Sciences (NIEHS), exposure to the metabolic toxin rotenone, a broad-spectrum pesticide used in agriculture, has been identified as a risk factor for the development of Parkinson's disease. We exposed mice to low doses of rotenone orally, using intragastric gavage, 5 days per week, from 6-12 months of age. Using this model system, we observed classical

behavioral and histopathologic symptoms of Parkinsonism that developed progressively over a six month time period. To determine if rotenone caused inflammatory changes in the CNS, we evaluated freshly prepared brain cells from mice that had been exposed to rotenone or vehicle for 6 months. We observed the expected CD45lo (resident microglia) and rare CD45hi (peripheral leukocyte) populations in our preparations and found that rotenone specifically increased the percentages of CD45hi cells as compared to vehicle treated mice. Although we observed evidence of microglial activation in histologic samples, co-labeling for the monocyte and microglial marker CD11b along with CD45 indicated that there was no change in the overall percentages of CD45lo/CD11b+ cells in rotenone treated mice, however, significant increases in CD45hi/CD11b- cells were observed as the result of rotenone exposure. We concluded that rotenone exposure led to an infiltration of peripheral, non-monocytic leukocytes into the CNS. We obtained striatal tissue extracts from an independent cohort of mice that had only been exposed to rotenone for 3 months, prior to the development of behavioral symptomology. We conducted an unbiased cytokine screen and observed a significant induction of pro-inflammatory cytokines in the brains of rotenone treated animals. Administration of the anti-inflammatory phosphodiesterase 4 - inhibitor rolipram at the 3-month time point inhibited the development of motor symptoms. Post-mortem analysis revealed a complete inhibition of leukocyte infiltration into the CNS of rotenone treated mice receiving rolipram. These findings indicate that detectable, reversible neuroinflammation can occur as a result of occupational exposure to pesticides. Findings will inform the development of preventative anti-inflammatory treatments for neurologic disorders in at risk populations such as the elderly, agricultural workers and military personnel.

Disclosures: M.C. Havrda: None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.10

Topic: C.03. Parkinson’s Disease

Title: Caspase inhibition mitigates alpha-synuclein cytotoxicity and mitochondrial demise in human dopaminergic neurons

Authors: *G. K. GANJAM[1], A. M. DOLGA[1], K. BOLTE[2], S. NEITEMEIER[1], M. HÖLLERHAGE[3], W. E. OERTEL[4], G. U. HOEGLINGER[3], C. CULMSEE[1];

[1]Philipps Univ. of Marburg, Marburg, Germany; [2]Cell Biol., Dept. of Biol., Marburg, Germany; [3]German center for Neurodegenerative Dis., Munich, Germany; [4]Dept. of Neurol., Marburg, Germany

Abstract: Parkinson's disease is a common neurodegenerative movement disorder characterized by dopaminergic neuronal loss in the substantia nigra that has been linked to α-synuclein toxicity. The molecular mechanisms underlying α-synuclein accumulation, agglomeration and toxicity in human dopaminergic neuronal loss are poorly defined. Hence, the goal of this study was to investigate the deleterious effects of α-synuclein in human dopaminergic Lund human mesencephalic (LUHMES) cells. In particular, we investigated a variant of α-synuclein protein targeted to mitochondria, since rapidly evolving concepts suggest a particular role of α-synuclein toxicity at the level of mitochondria in PD. Therefore, we have engineered novel adeno-associated virus type-2 based models for α-synuclein protein expression in the cytosol or in mitochondria. Overexpression of cytosolic and the mitochondrial variants of α-synuclein severely disrupted the dendritic network, induced loss of cellular ATP, enhanced mitochondrial ROS production, and was associated with activation of caspases and dopaminergic cell death in a time-dependent manner. In addition, real-time analysis of mitochondrial bioenergetics using Seahorse Bioscience system following AAV infection elicited a complete damage to mitochondrial respiration capacity in dopaminergic neurons. Mitochondrial targeted expression of α-synuclein appears to be more toxic than the cytosolic form of α-synuclein. In addition, ultrastructural mitochondrial morphological analysis by transmission electron microscopy illustrated a number of deformed cristae in cytosolic form and a complete loss of cristae structure and massively swollen mitochondria after expression of mitochondrial targeted α-synuclein in the human dopaminergic neurons. Furthermore, we addressed the question whether dopaminergic neuronal cell death induced by α-synuclein could be rescued by pharmacological approaches. We found that inhibition of caspases by QVD significantly ameliorated α-synuclein induced dopaminergic neuronal death. Interestingly, inhibition of caspases preserved neuronal network integrity, ATP levels and mitochondrial respiration capacity in both paradigms of cytosolic and mitochondrial α-synuclein overexpression. Overall, our findings show that cytosolic as well as mitochondrial targeted expression of α-synuclein is detrimental to human dopaminergic neurons, and inhibition of caspases amend α-synuclein toxicity. Thus, caspase inhibitors provide promising therapeutic potential to prevent dopaminergic neuronal death in Parkinson's syndromes that are associated with α-synuclein toxicity.

Disclosures: G.K. Ganjam: None. **A.M. Dolga:** None. **K. Bolte:** None. **S. Neitemeier:** None. **M. Höllerhage:** None. **W.E. Oertel:** None. **G.U. Hoeglinger:** None. **C. Culmsee:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.11

Topic: C.03. Parkinson's Disease

Support: Parkinson's UK Grant G-1203

Title: Rab11 modulates alpha-synuclein mediated defects in synaptic transmission and behaviour

Authors: *F. GIORGINI[1], C. BREDA[1], M. L. NUGENT[2], J. G. ESTRANERO[1], C. P. KYRIACOU[1], T. F. OUTEIRO[3], J. R. STEINERT[2];
[1]Dept. of Genet., Univ. of Leicester, Leicester, United Kingdom; [2]MRC Toxicology Unit, Leicester, United Kingdom; [3]Univ. of Goettingen, Goettingen, Germany

Abstract: A central pathological hallmark of Parkinson's disease (PD) is the presence of proteinaceous depositions known as Lewy bodies, which consist largely of the protein alpha-synuclein (aSyn). Mutations, multiplications, and polymorphisms in the gene encoding aSyn are associated with familial forms of PD and susceptibility to idiopathic PD. Alterations in aSyn impair neuronal vesicle formation/transport, and likely contribute to PD pathogenesis by neuronal dysfunction and degeneration. aSyn is functionally associated with several Rab family GTPases, which perform various functions in vesicle trafficking. Here we explore the role of Rab11 - which is critical in endosomal recycling - in the pathogenesis of PD using Drosophila models of aSyn toxicity. We find that aSyn potentiates synaptic transmission at the larval neuromuscular junction by increasing synaptic vesicle size, and that these alterations are reversed by Rab11. Furthermore, Rab11 ameliorates several aSyn-dependent phenotypes in both larvae and adult fruit flies, including locomotor activity, degeneration of dopaminergic neurons, and shortened lifespan. This work highlights the importance of Rab11 in aSyn-dependent defects, particularly in the modulation of synaptic dysfunction due to changes in synaptic vesicle size. Notably, our data suggest that targeting Rab11 activity may have unexplored therapeutic value in PD.

Disclosures: **F. Giorgini:** None. **C. Breda:** None. **M.L. Nugent:** None. **J.G. Estranero:** None. **C.P. Kyriacou:** None. **T.F. Outeiro:** None. **J.R. Steinert:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.12

Topic: C.03. Parkinson's Disease

Support: EXPL/BIM-MED/0822/2013

SFRH/BD/90365/2012

Title: MicroRNA-124 loaded nanoparticles modulate neurogenesis in the subventricular zone

Authors: *C. M. SARAIVA[1], J. M. PAIVA[2], L. FERREIRA[3,2], L. I. BERNARDINO[4]; [1]Fac. of Hlth. Sci., Hlth. Sci. Res. Ctr. - Univ. of Be, Covilhã, Portugal; [2]Biocant, Ctr. of Innovation in Biotech., Cantanhede, Portugal; [3]Ctr. for Neurosci. and Cell Biol., Coimbra, Portugal; [4]Hlth. Sci. Res. Ctr., Covilhã, Portugal

Abstract: The subventricular zone (SVZ) lining the lateral ventricles comprises the largest population of neural stem cells (NSCs) in the adult mammalian brain. NSCs are multipotent and can give rise to neurons and glia cells. MicroRNA (miR)-124 has been recently described to trigger neuron commitment of NSCs. However, current strategies to deliver miRs into cells or tissues are not efficient. Thus, identifying new platforms to deliver proneurogenic molecules such as miR124 is crucial to boost neurogenesis and to take advantage of the huge potential of endogenous NSCs to repair the damaged brain. The main goal of this work is to study the inductive effect of miR-124-loaded nanoparticles (miR-124 NPs) in the differentiation of NSCs into new neurons. For this purpose, neonatal P1-3 C57BL/6 mice were used to obtain stem/progenitors cell cultures from the SVZ. The cells were grown as neurospheres for 5 days and then seeded on coverslips and allowed to adhere. The resultant cell monolayer was then transfected with several concentrations (1, 10 and 20 μg/mL) of NPs complexed with 200 nM of miR-124. We found that 1 μg/mL of NPs did not interfere with cell toxicity (accessed by propidium iodide and TUNEL assays) or proliferation (BrdU assay). Interestingly, 1μg/mL of NPs complexed with miR-124 was able to increase the differentiation into neurons (NeuN-immunoreactivity) in about 25% compared with non-treated (controls) or void NPs treated cultures. Additionally, no change in the oligodendrocyte commitment was observed (olig2-immunoreactivity). The relative mRNA amount of two validated miR-124 targets, sox9 and jagged1, was also assessed by qPCR. As expected, miR-124 NPs reduced the expression of both genes as compared with controls. Moreover, miR124 NPs induced a significant decrease in the number of sox9-immunoreactive positive cells (approximately 20%) as compared with controls. Taken together, our results showed that the presence of miR-124 delivered by NPs increase the neuronal commitment of SVZ stem/progenitors cells, being the 1μg/mL NPs - 200 nM miR-124 the most suitable formulation. These results provide clear evidences to support the use of miR-124 NPs as a new therapeutic approach to boost brain repair endogenous mechanisms in the setting of neurodegenerative diseases.

Disclosures: **C.M. Saraiva:** None. **J.M. Paiva:** None. **L. Ferreira:** None. **L.I. Bernardino:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.13

Topic: C.03. Parkinson's Disease

Support: GUMC Funds

Title: Towards a novel therapy for synucleinopathies: Candesartan cilexetil inhibits oligomeric alpha-synuclein-induced neuroinflammation

Authors: ***S. G. DANIELE**, K. MAGUIRE-ZEISS;
Neurosci., Georgetown Univ. Med. Ctr., Washington, DC

Abstract: Synucleinopathies, such as Parkinson's disease, are progressive neurodegenerative disorders characterized by the loss of selective neurons and the accumulation of oligomeric α-synuclein in neuronal cell bodies and neurites. Importantly, glial activation is present at both early and late stages of these disorders, indicating a molecular interplay between neuroinflammation and disease progression. We have demonstrated that oligomeric synuclein induces complex morphofunctional changes in primary microglia including a shift to ambeoid morphology, enhanced nuclear translocation of the NFkB p65 subunit, and increased expression of proinflammatory molecules and toll-like receptors. In addition, this activation is mediated through the toll-like receptor adaptor protein, MyD88. Here we demonstrate that oligomeric synuclein activates microglia by directly interacting with the toll-like receptor heterodimer TLR1/2 at the cell's surface. We further show that the FDA-approved angiotensin II receptor blocker, candesartan cilexetil, attenuates synuclein-induced microglial activation. In our paradigm, the anti-inflammatory effects of candesartan are evidenced by a decrease in the biochemical and morphological immunophenotype of activated microglia, devoid of a concurrent increase in molecules indicative of immune-resolution and repair, such as IL-10. Since microglia do not express angiotensin II receptors and oligomeric synuclein directly engages TLR1/2, we hypothesize that candesartan acts antagonistic to the TLR1/2 signaling pathway. Our work supports the further development of therapeutics directed at attenuating the neuroinflammatory response in synucleinopathies.

Disclosures: **S.G. Daniele:** None. **K. Maguire-Zeiss:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.14

Topic: B.02. Ligand-Gated Ion Channels

Title: Hybrid nanostructures for exclusive inhibition of extrasynaptic NMDA receptors

Authors: E. MOLOKANOVA[1], G. H. BRAUN[2], *A. SAVTCHENKO[3];
[1]Nanotools Biosci., Encinitas, CA; [2]Sanford Burnahm Med. Res. Inst., La Jolla, CA; [3]Univ. of California - San Diego, La Jolla, CA

Abstract: Brain disorders take a heavy economic and social toll on our society. Glutamatergic cytotoxicity mediated by overactivation of NMDA receptors (NMDARs) is implicated in many neurological disorders, including ischemic stroke, brain trauma, amyotrophic lateral sclerosis, Alzheimer's, Parkinson's, and Huntington's diseases. To be therapeutically viable, NMDAR antagonists must block only excessive pathological activation of receptors, while preserving their normal physiological role in synaptic neurotransmission. Here we report a novel NMDAR antagonist that satisfies this two-fold requirement. Given that synaptic NMDARs (sNMDARs) support physiological processes and extrasynaptic NMDARs (eNMDARs) mediate pathological pathways, we decided to design eNMDAR-specific antagonists by exploiting differences in spatial characteristics of subcellular locations of sNMDARs and eNMDARs. Here we present a nanostructure comprising the NMDAR antagonist attached via PEG polymers to a gold (Au) nanoparticle (Au-Memantine). This nanostructure engineered to be larger than the synaptic cleft was capable of efficient and selective inhibition of eNMDARs, while having no effect on sNMDARs and synaptic transmission in cerebrocortical neurons. Furthermore, Au-Memantine was able to prevent dendritic spine loss triggered by Aβ oligomers in organotypic hippocampal slices, and was more effective than free memantine. The ability to manipulate eNMDAR-mediated pathways is crucial both for understanding the mechanisms of neurological disorders and for development of rational approaches for pharmacological treatments. The advantage of proposed nanostructures is that all three components (memantine, gold, and PEG) are approved by FDA for use in humans. Due to its size and remarkable pharmacological properties, Au-Memantine can discriminate between different NMDAR-mediated pathways responsible for normal and pathological brain activities, thus ensuring potentially improved clinically

tolerability. Our results could have far-reaching implications in both basic and translational neuroscience as this study provides proof-of-concept for a new class of neuroprotective drugs for a wide spectrum of neurological disorders with a dichotomic synaptic vs. extrasynaptic activity pattern.

Disclosures: E. Molokanova: None. **G.H. Braun:** None. **A. Savtchenko:** None.

Nanosymposium

109. Astrocyte Action in CNS Disorders

Location: 144A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 109.01

Topic: B.11. Glial Mechanisms

Support: Guthy-Jackson Charitable Foundation

Mayo Clinic Department of Neurology

Title: Astrocytes orchestrate early granulocyte responses in neuromyelitis optica

Authors: *M. CAULFIELD, T. KAPTZAN, R. LAFRANCE-COREY, Y. GUO, C. HOWE, C. LUCCHINETTI;
Mayo Clin., Rochester, MN

Abstract: Neuromyelitis optica (NMO) is a CNS inflammatory disorder in which astrocytes are selectively targeted through the binding of a pathogenic, complement-activating IgG autoantibody (NMO-IgG) to the ectodomain of aquaporin-4 (AQP4). AQP4 is the principal CNS water channel where it is expressed on astrocytic foot processes at astro-endothelial, astro-pial, and astro-neuronal synapses. Detection of the NMO-IgG autoantibody in serum unifies a growing spectrum of clinical disorders known as NMO spectrum disorders (NMOSD), which include optic neuritis, transverse myelitis, intractable vomiting and hiccups, dysphagia, inappropriate anti-diuresis, central hypotension, oculomotor dysfunction, hearing loss, narcolepsy, central endocrinopathies, posterior reversible encephalopathy, and generalized encephalopathy. Our observations in NMO tissues demonstrate that the disease is a global astrocytopathy with evidence of an early and robust astrocytic stress response, frequently within regions of tissue not associated with overt demyelination. We also find eosinophils and other granulocytes near reactive astrocytes, in the absence of complement deposition, suggesting that these cells have been recruited to the CNS parenchyma through a distinct mechanism. Upon

activation, astrocytes can synthesize many immunomodulatory and immunopathogenic cytokines and chemokines. Therefore, we hypothesize that astrocytes orchestrate early granulocytic recruitment to the CNS in NMO. Using primary murine astrocyte cultures we have found that stimulation with NMO-IgG drives a robust pro-inflammatory, pro-granulocytic response at the transcript level via microarray analysis, as well as the release of large amounts of the granulocytic chemokines CCL5, CXCL1, and CXCL2. Further, in vivo granulocytes rapidly accumulate in the brain following intracranial NMO-IgG injection. Although the consequences of early granulocyte infiltration in NMO are largely unknown it is likely that they contribute significantly to tissue damage. We hypothesize that astrocytes act at the nexus of brain-immune interactions to coordinate this trafficking. Further, we believe that granulocytes are obligate effectors in NMO tissue and represent a novel and powerful target for therapeutic intervention, likely before irreversible tissue destruction has occurred.

Disclosures: **M. Caulfield:** None. **T. Kaptzan:** None. **R. LaFrance-Corey:** None. **Y. Guo:** None. **C. Howe:** None. **C. Lucchinetti:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); named inventor on patents (12/678,350 filed 2010 and 12/573,942 filed 2008) and shares in royalties from patent (#7101679 issued 2006).

Nanosymposium

109. Astrocyte Action in CNS Disorders

Location: 144A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 109.02

Topic: B.11. Glial Mechanisms

Title: Sustained downregulation of β-dystroglycan in astrocytic endfeet in epileptic cerebral cortex

Authors: ***A. GONDOU**, T. SHINOTSUKA, A. MORITA, M. YASUI, M. NURIYA; Pharmacol., Keio Univ., Tokyo, Japan

Abstract: Epilepsy is characterized by the abnormal activation of neurons in the cerebral cortex, but the molecular and cellular mechanisms contributing to the development of recurrent seizures are largely unknown. Recently, the critical involvement of astrocytes in the pathophysiology of epilepsy has been proposed. However, the nature of plastic modulations of astrocytic proteins in the epileptic cortex remains poorly understood. In this study, we utilized the zero magnesium in vitro model of epilepsy and examine the potential molecular changes of cortical astrocytes

focusing specifically on endfeet where specialized biochemical compartments exist. We find that continuous epileptic activation of neurons for 1 hour decrease the expression level of β-dystroglycan (βDG) in acute cortical brain slices prepared from mice. This change is completely abolished by the pharmacological blockade of NMDA-type glutamate receptors as well as MMP inhibitors. Consistent with the specific localization of βDG at astrocytic endfeet where it plays a pivotal role in anchoring endfeet -enriched proteins in astrocytes, the downregulation of βDG is accompanied by a decrease in the expression of AQP4 but not laminin. Importantly, this downregulation of βDG persists for at least 1 hour, even after the apparent recovery of neuronal activation. Finally, we show that this downregulation of βDG is associated with the impairment of the diffusion blockade function of endfeet at the blood-brain interface. These results suggest that sustained βDG dysregulation at astrocytic endfeet occurs in the epileptic cerebral cortex and may contribute to the pathogenesis of epilepsy.

Disclosures: **A. Gondou:** None. **T. Shinotsuka:** None. **A. Morita:** None. **M. Yasui:** None. **M. Nuriya:** None.

Nanosymposium

109. Astrocyte Action in CNS Disorders

Location: 144A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 109.03

Topic: B.11. Glial Mechanisms

Support: 973 Program 2011CB504400 and 2012CB966900

Shenzhen Governmental Basic Research Grant JC201006040897A, JC201005270296A, and JC201005270291A

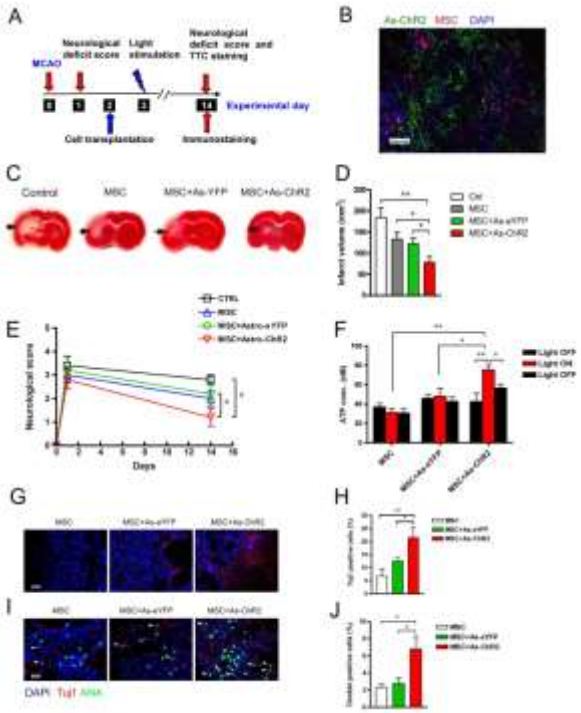
Shenzhen Peacock Plan KQCX20130628112914294

NSFC Grant 91132306

Title: Optogenetic stimulation of astrocytes promotes neuronal differentiation of mesenchymal stem cells and improves the neurological deficit in stroke rats

Authors: ***J. TU**, F. YANG, Y. LIU, P. WEI, L. WANG;
Shenzhen Key Lab. of Neuropsychiatric Modulation, Shenzhen Inst. of Advanced Technol., Guangdong, China

Abstract: Astrocytes have been identified as key components of the stem cell niche. However, it is not clear whether astrocyte-derived ATP plays a vital role in modulating the function of mesenchymal stem cells (MSCs). Herein, we, for the first time co-cultured MSCs with light-stimulated-channelrhodopisn-2 (ChR2)-astrocytes, and observed these MSCs expressed more neuronal markers, Tuj1 and NeuN. Furthermore, the ChR2-astrocyte-conditioned medium markedly up-regulated mRNA expression of Tuj1 and Pax6, and promoted the DNA synthesis of MSCs, indicating some component(s) from the photostimulated ChR2-astrocytes contributed to the differentiation- and proliferation- enhancing effects. Optical stimulation of ChR2-astrocytes significantly increased ATP accumulation in their bathing medium without impairing the cell membrane. We further demonstrated the enhancing effects of ATP on the MSCs through the wnt/beta-catenin signalling in a dose-dependent manner. Furthermore, either FZD8 or beta-catenin mRNA level was significantly increased by ATP, and this effect could be reversed by application of the selective P2X receptor antagonist, TNP-ATP. Together these data provide convergent evidence that ATP from photostimulated- astrocytes, through binding to the P2X receptors expressed by MSCs, activates the wnt/beta-catenin signalling, and as a consequence, upregulates neuronal differentiation of MSC, thereb providing a molecular mechanism for modulation of stem cell function by activated-astrocytes within a special niche. Finally but importantly, our study also demonstrated that light-controlled astrocytes stimulated endogenous ATP release into the ischemic area to influence the transplanted MSC-niche, resulting in steering the MSCs towards neuronal differentiation and improvements of neurological deficit in the stroke rats.



Disclosures: **J. Tu:** None. **F. Yang:** None. **Y. Liu:** None. **P. Wei:** None. **L. Wang:** None.

Nanosymposium

109. Astrocyte Action in CNS Disorders

Location: 144A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 109.04

Topic: B.11. Glial Mechanisms

Support: Wellcome Trust

Medical Research Council

Title: Are astrocytes the hypoxia sensors of the central nervous system?

Authors: *I. N. CHRISTIE[1], P. R. ANGELOVA[2], V. KASYMOV[3], A. Y. ABRAMOV[2], A. V. GOURINE[3];
[1]Ctr. for Advanced Biomed. Imaging, [2]Inst. of Neurol., [3]Univ. Col. London, London, United Kingdom

Abstract: Highly specialized peripheral oxygen sensing elements evolved to monitor and ensure adequate oxygenation of the arterial blood supplying the brain. Located in the carotid sinus and the aortic arch, peripheral respiratory chemoreceptors detect decreases in PO_2 and trigger adaptive changes in breathing. However, the organism survives complete loss of peripheral oxygen chemosensitivity pointing to the existence of functional oxygen sensors in the central nervous system. Astrocytes are widely believed to regulate neurovascular tone and mediate increases in blood flow upon increased neuronal activity. Here we tested the hypothesis that the ubiquitous astrocyte is sensitive to physiological changes in brain oxygenation. Using 2-photon imaging *in vivo* (anesthetized and artificially ventilated rats, n=9) we detected waves of $[Ca^{2+}]_i$ responses in cortical astrocytes when the concentration of oxygen in the inspired air was lowered for 60 s from 21% to 15% or 10%. No calcium responses were observed in neurons, while arteriole dilation was observed during hypoxia. Measurements of tissue oxygenation revealed that during acute exposure to 15 and 10% O_2 in the inspired air, parenchymal PO_2 in the rat cerebral cortex decreased from 24±3 to 11±2 and 6±1 mmHg, respectively (n=6). *In vitro* removal of external Ca^{2+} had no effect on hypoxia-evoked Ca^{2+} signalling in astrocytes while application of SERCA inhibitor thapsigargin completely blocked the responses. Cellular mechanism of PO_2 sensing involves activation of the PLC pathway as demonstrated in the experiments showing blockade of the hypoxia-induced responses by inhibitors of PLC downstream processes (U73122, 2-APB or Xestospongine C). Hypoxia-induced $[Ca^{2+}]_i$ responses were blocked by depolarization of astroglial mitochondria using uncoupler FCCP and, therefore, appear to be dependent on the mitochondrial membrane potential. These data suggest

that astrocytes are sensitive to physiological changes in PO_2 and this sensitivity may have a functional significance in the control of local blood flow and the neuronal activity.

Disclosures: I.N. Christie: None. **P.R. Angelova:** None. **V. Kasymov:** None. **A.Y. Abramov:** None. **A.V. Gourine:** None.

Nanosymposium

109. Astrocyte Action in CNS Disorders

Location: 144A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 109.05

Topic: B.11. Glial Mechanisms

Support: The Sällskapet Barnavård Foundation

The regional agreement on medical training and clinical research (ALF) between Stockholm County Council and Karolinska Institutet

Wera Ekström Foundation

Title: Reduced glutamate uptake in astrocytes expressing the Na,K-ATPase α2 mutation G301R. Implications for Familial Hemiplegic Migraine type 2

Authors: *E. GUNNARSON[1], N. ILLARIONOVA[1], P. BØTTGER[2], Y. SONG[3], A. APERIA[1], K. LYKKE-HARTMANN[2];
[1]Karolinska Institutet, Stockholm, Sweden; [2]Aarhus Univ., Aarhus, Denmark; [3]Shanghai Clin. Res. Ctr., Shanghai, China

Abstract: The neurological disorder familial hemiplegic migraine type 2 (FHM2) is linked to mutations in the catalytic α2 isoform of Na,K-ATPase. The mechanisms behind the symptoms caused by α2 mutations are not well understood. In the adult brain α2 is expressed in astrocytes together with the ubiquitous α1 isoform. One of the essential functions of astrocytes is glutamate uptake from the extra-synaptic space following neuronal activity. Glutamate uptake is to a large extent driven by the trans-membrane sodium gradient created by active sodium transport via the Na,K-ATPase. We have determined α1 and α2 abundance and glutamate uptake, using tritium labeled aspartate uptake (^{3}H D-Asp) as an index for glutamate uptake, in primary cultures from E17 hippocampus of wild type ($\alpha2^{+/+}$), heterozygous ($\alpha2^{+/G301R}$) and homozygous ($\alpha2^{G301R/G301R}$) α2 mutant mice carrying the knock-in FHM2-mutation G301R. The Na,K-ATPase α2 protein abundance was reduced in the $\alpha2^{G301R/G301R}$ hippocampal cells compared to WT $\alpha2^{+/+}$ cells.

Cultures from $\alpha2^{+/+}$, $\alpha2^{+/G301R}$ and $\alpha2^{G301R/G301R}$ mice all expressed similar levels of the Na,K-ATPase α1 subunit and Na^+-coupled glutamate transporter GLAST. The D-asp uptake was significantly reduced in astrocytes expressing the mutant α2 and lower in $\alpha2^{G301R/G301R}$ (-17%) than in $\alpha2^{+/G301R}$ cells (-11%). When Venus-tagged WT or mutant α2 was transfected into an astrocyte cell line and WT primary astrocytes, the plasma membrane expression of the mutant G301R α2 was reduced in comparison to WT α2. The results indicate that reduced capacity of astrocytes to take up glutamate and temporary increases in ambient glutamate concentration may be implicated in the symptomatology of FHM2 associated with α2 mutations.

Disclosures: E. Gunnarson: None. **N. Illarionova:** None. **A. Aperia:** None. **K. Lykke-Hartmann:** None. **P. Bøttger:** None. **Y. Song:** None.

Nanosymposium

109. Astrocyte Action in CNS Disorders

Location: 144A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 109.06

Topic: B.11. Glial Mechanisms

Support: NIH P01-HL2095488

NCRR P30RR032135

NIGMS P30GM103498

Totman Medical Research Trust Fund

Peter Martin Aneurysm Endowment

S10 OD 010583

Title: Increased amplitude of spontaneous Ca2+ events in astrocytic endfeet underlies inversion of neurovascular coupling after subarachnoid hemorrhage

Authors: *A. C. PAPPAS, M. KOIDE, G. C. WELLMAN;
Dept. of Pharmacol., Univ. of Vermont, Burlington, VT

Abstract: Increased local cerebral blood flow (CBF) and associated increases in oxygen delivery are crucial for maintaining neuronal function and survival in active brain regions. The process by which local CBF is dynamically regulated to meet the ongoing metabolic demand of active

neurons is called functional hyperemia or neurovascular coupling (NVC). Briefly, NVC in the healthy brain involves: 1) increased synaptic transmission, 2) a propagating wave of Ca^{2+} in associated astrocytes that terminates in the perivascular endfeet, 3) Ca^{2+}-dependent release of vasodilatory substances (e.g. K^+ efflux via large conductance Ca^{2+}-activated K^+ [BK] channels) from the endfeet onto the underlying arteriole and 4) arteriolar dilation and increased local CBF. Recently, inversion of NVC from vasodilation to vasoconstriction was demonstrated in brain slices obtained from subarachnoid hemorrhage (SAH) model rats (Koide *et al.* PNAS 109: E1387-E1395, 2012). This pathological response, which could restrict blood flow to active brain regions, coincided with the increased amplitude of spontaneous Ca^{2+} events in astrocytic endfeet. Here, our goal was to provide evidence of a causal link between these two phenomena. The inversion of NVC and high amplitude spontaneous endfoot Ca^{2+} events were first observed in brain slices (≈ 70 % of slices) from animals 24 hr post-SAH. At 48 hrs and 96 hrs post-SAH, nearly all brain slices exhibited inversion of NVC and high amplitude spontaneous Ca^{2+} events. Additional studies were performed measuring both NVC and spontaneous Ca^{2+} activity in continuous recordings from brain slices using 24 hr SAH rats, where only EFS-induced dilation or constriction was observed in a given brain slice. All brain slices exhibiting EFS-induced vasoconstriction were accompanied by high-amplitude spontaneous Ca^{2+} events in the surrounding endfeet, whereas only low-amplitude events were observed around vessels that dilated. Further, to mimic EFS-induced K^+ release through endfoot BK channels, we raised the extracellular K^+ concentration ($[K^+]_o$) from 3 mM to 10 mM in the brain slice superfusate. As expected, this modest elevation of $[K^+]_o$ caused vasodilation in brain slices from control animals, whereas it caused constriction after SAH, consistent with SAH-induced inversion of NVC. Interestingly, pharmacologic depletion of intracellular Ca^{2+} stores, which abolished all spontaneous Ca^{2+} activity in the endfeet, restored arteriolar dilation to a modest elevation of $[K^+]_o$ in brain slices from SAH animals. Together, our data demonstrate a key role for the increased amplitude of spontaneous Ca^{2+} events in astrocytic endfeet in causing inversion of NVC after SAH.

Disclosures: **A.C. Pappas:** None. **M. Koide:** None. **G.C. Wellman:** None.

Nanosymposium

109. Astrocyte Action in CNS Disorders

Location: 144A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 109.07

Topic: B.11. Glial Mechanisms

Support: NIH Grant MH094268

Title: Astrocytic ALDH7A1 pathophysiology in neuropsychiatric disorders

Authors: *T. E. FAUST[1,2], H. HIYAMA[3], S. ZOUBOVSKY[1], H. JAARO-PELED[1], K. NI[3], A. SAWA[1];
[1]Psychiatry, [2]Neurosci., Johns Hopkins Univ. SOM, Baltimore, MD; [3]Astellas Pharma Inc., Tsukuba-shi, Japan

Abstract: The function of astrocytes as supporting cells in the brain has long been recognized. However, astrocytes have increasingly been shown to affect neuronal signaling through glutamate uptake, release of gliotransmitters, regulation of extracellular K^+ levels and maintenance of osmotic balance. Disruption of astrocyte function plays an important role in several central nervous system (CNS) disorders. Recent studies have reported that aldehyde dehydrogenase 7a1 (ALDH7A1; also antiquitin) is expressed 10-fold higher in astrocytes compared to other CNS cell types, although its function in astrocytes remains unknown. In non-CNS cells, ALDH7A1 plays a central role in the lysine degradation pathway. It also has been shown to protect against certain cell stressors including osmotic pressure and reactive oxygen species (ROS). Clinical studies have identified mutations in the human *ALDH7A1* gene as the primary cause of pyridoxine-dependent epilepsy (PDE), a childhood form of epilepsy treated with high doses of pyridoxine. The loss of ALDH7A1 enzymatic activity in these patients results in buildup of the toxic intermediate piperideine-6-carboxylate which reacts with pyridoxal phosphate (PLP), depleting the brain of an important co-factor required for more than 140 enzymatic reactions. We found that ALDH7A1 is highly expressed in multiple astrocyte subpopulations in the adult mouse CNS. We have also found that expression of ALDH7A1 is decreased in tissues from patients with schizophrenia (SZ) and in a mouse model of psychosis (PCP). Clinical evidence is suggestive of co-morbidity between SZ and epilepsy; functionally, these diseases are linked by alterations in neuronal excitability. In SZ, cognitive changes include working memory (WM) and/or frontal functional deficits influenced by oxidative stress. To study how decreased expression of ALDH7A1 may affect signaling in the brain, we have generated a conditional knockout (cKO) mouse containing a floxed allele of *Aldh7a1*. We found that *Aldh7a1 null* mice have increased levels of oxidative stress compared to wild type (WT) mice. For further analysis this protein's function, we have crossed the floxed mice with *GLAST-CreER* mice that express Cre specifically in astrocytes (*Aldh7a1* cKO^{GLAST} mice). We therefore hypothesize that reduced expression of ALDH7A1 renders astrocytes more susceptible to oxidative and osmotic stress, thus disrupting their ability to effectively modulate synaptic signaling.

Disclosures: **T.E. Faust:** None. **H. Hiyama:** A. Employment/Salary (full or part-time):; Astellas Pharma Inc.. **S. Zoubovsky:** None. **H. Jaaro-Peled:** None. **K. Ni:** A. Employment/Salary (full or part-time):; Astellas. **A. Sawa:** None.

Nanosymposium

109. Astrocyte Action in CNS Disorders

Location: 144A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 109.08

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Swebilius Award 2014

Title: Branched chain amino acids in post stroke epileptogenesis

Authors: *E. C. DAMISAH[1], E. PEREZ[1], H. ZAVERI[2], D. SPENCER[1], T. EID[1];
[1]Neurosurg., Yale Univ., New Haven, CT; [2]Neurosurg., Yale Univ., New haven, CT

Abstract: Approximately 10% of human patients who survive a stroke develop epilepsy (Myint, Staufenberg et al. 2006). However, the cellular, molecular and physiological mechanisms underlying post-stroke epileptogenesis are not well understood. It is particularly important to study the epileptogenic period because it represents a unique window where the process that leads to epilepsy may be halted or modified (yudkoff 1997). Using *in vivo* microdialysis studies of human patients with epilepsy, our lab has shown that glutamate (During and Spencer 1993) and branched chain amino acids (BCAA) levels are significantly increased in the epileptogenic cortex at baseline and before seizure onset (unpublished data). Here we test the hypothesis that BCAAs are increased in the brain extracellular fluid of rats that will go on to develop epilepsy after a small right parietal stroke in the photo cortical thrombosis model of epilepsy. Additionally, specific electroencephalography (EEG) characteristics are predictive of future development of epilepsy during the epileptogenic period. A total of 16 male rats with right parietal strokes of equal volume underwent continuous EEG one week after stroke induction until first electrographic and/or clinical seizure. About 18% (3) animals with stroke developed spontaneous recurrent seizures, starting about eight weeks after stroke induction. These animals also had EEG characteristics: spike and wave discharge patterns that were predictive of future seizures during the weeks leading to first seizure compared to animals who did not develop epilepsy even though they had the same volume and location of stroke. The rats that have abnormal spike wave discharges (which are predictive of future seizures) will also undergo *in vivo* micro dialysis and based on our prior results, it is expected that these rats will have increased BCAA levels compared to the rats that do not develop seizure. This is significant as it suggests that there might be chemical and EEG markers predictive of epilepsy after stroke and sheds some light on the pathophysiology of epileptogenesis

Disclosures: **E.C. Damisah:** None. **E. Perez:** None. **H. Zaveri:** None. **D. Spencer:** None. **T. Eid:** None.

Nanosymposium

109. Astrocyte Action in CNS Disorders

Location: 144A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 109.09

Topic: B.11. Glial Mechanisms

Support: NIH Grant 5R01AA018799

Title: Astrocyte regulation of ethanol tolerance

Authors: ***S. PARKHURST**, P. ADHIKARI, F. W. WOLF;
Univ. of California, Merced, Merced, CA

Abstract: Increased alcohol intake is facilitated by the development of tolerance, a simple form of behavioral plasticity, most basically defined as the acquired resistance to the aversive effects of the drug. Through previous research in Drosophila, the fruit fly, a glial-specific gene was discovered that is required for the development of ethanol tolerance, demonstrating that glia regulate ethanol responses. One subset of glia, astrocytes, are the primary regulators of glutamate homeostasis at synapses in mammals and regulate functional properties of neurons. Fly astrocytes are thought to perform many of the same roles as their vertebrate counterparts, being located in close proximity to synapses and sharing a similar morphology. To determine the role of astrocytes in the development of ethanol tolerance, we performed a survey of known astrocyte functions. Genetic manipulations were performed using the GAL4/UAS system in adult flies to uncover ethanol sensitivity and tolerance phenotypes. The astrocyte properties that were manipulated include: vesicular recycling, calcium signaling, and the maintenance of glutamate homeostasis. Our findings suggest that there is a pathway involved in vesicular release but not dynamin-dependent endocytosis which regulates ethanol tolerance. There is also a calcium-dependent pathway in astrocytes that does not require calcium from the endoplasmic reticulum, but does utilize calmodulin, to regulate ethanol tolerance. Furthermore, interruption of broad G-protein coupled receptor signaling suggests that extracellular communication by neuromodulators promotes tolerance. Most notably we found that decreased expression of the plasma membrane-bound glutamate transporter in astrocytes, EAAT1 (Glt-1 in mice), causes markedly increased sedation sensitivity and decreased ethanol tolerance, without causing any observable neurodegeneration. The work described above demonstrates that astrocytes actively

regulate ethanol tolerance and lays the groundwork for future studies into a more precise mechanism explaining how.

Disclosures: S. Parkhurst: None. **P. Adhikari:** None. **F.W. Wolf:** None.

Nanosymposium

109. Astrocyte Action in CNS Disorders

Location: 144A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 109.10

Topic: B.11. Glial Mechanisms

Support: NIH Grant

Title: Astrocytic swelling-mediated increases in neuronal excitability: implications for cerebral edema and epilepsy

Authors: *K. LAUDERDALE, T. R. MURPHY, D. K. BINDER, T. A. FIACCO;
Univ. of California Riverside, Riverside, CA

Abstract: Cerebral edema and seizures are associated with a plethora of diseases, disorders, and conditions such as traumatic brain injury, stroke, cardiac arrest, autism and epilepsy. Treatments for these conditions are often limited or ineffective, substantiating the need to further understand the cell-specific contributions and mechanisms involved. It has been known for some time that cell swelling and reduction of the extracellular space can lead to increases in neuronal excitability and even to seizures in vitro and in vivo. The elevated neuronal excitability in these conditions has been attributed to increased ephaptic interactions between neurons. However, astrocytes may contribute actively to this process due to their selective expression of the glial water channel aquaporin 4 (AQP4), together with evidence that astrocyte swelling in vitro leads to significant amounts of glutamate release through astrocytic volume-regulated anion channels (VRAC). Using electrophysiological whole-cell patch clamp techniques, currents and potentials were recorded in CA1 pyramidal neurons during application of hypoosmolar ACSF (hACSF) in acutely isolated slices from mice. Real-time astrocyte volume measurements indicated that application of hACSF rapidly and significantly swelled astrocytes within 1 minute of application. Astrocytic swelling evoked NMDA receptor-driven slow inward currents (SICs) in neurons, which, like astrocyte volume changes, initiated within 1 minute of hACSF application. Furthermore, both astrocyte volume changes and neuronal SICs were re-evokable with successive hACSF applications over matching timecourses. Neuronal excitability increased as

osmolarity decreased in a dose-dependent manner. Even 5% reductions in osmolarity were sufficient to significantly increase neuronal excitability. Blocking the NR2B subunit containing NMDA receptors with Ro 25-6981 decreased neuronal SICs during astrocyte swelling. In current clamp recordings, astrocyte swelling evoked neuronal action potentials (APs) in the absence or presence of the AMPA receptor antagonist NBQX. Furthermore, increased subthreshold EPSPs and neuronal APs were observed in the presence of Mg2+, suggesting that astrocyte swelling evokes increases in neuronal excitability in physiological conditions. Ongoing experiments will assay the effect of astrocytic AQP4 KO, glutamate loading of astrocytes, and astrocytic VRACs, further defining the mechanisms underlying astrocytic swelling increases in neuronal excitability. These studies could uncover several novel astrocytic targets and therapies to treat a vast range of CNS syndromes, diseases and disorders.

Disclosures: K. Lauderdale: None. **T.R. Murphy:** None. **D.K. Binder:** None. **T.A. Fiacco:** None.

Nanosymposium

109. Astrocyte Action in CNS Disorders

Location: 144A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 109.11

Topic: B.11. Glial Mechanisms

Support: the Families of SMA

the SMA Foundation

Title: Neuron- astrocyte interactions in synaptic activities in spinal muscular atrophy

Authors: *C. ZHOU, Z. FENG, C.-P. KO;
UNIVERSITY OF SOUTHERN CALIFORNIA, Los Angeles, CA

Abstract: Spinal Muscular Atrophy (SMA) is a neurodegenerative disease characterized by loss of synapses and motoneurons in the spinal cordand atrophy of skeletal muscles. An emerging concept suggests that many neurodegenerative diseases are non-cell autonomous, and glial cells are actively involved in the disease mechanisms. However, whether and how glial cells may contribute to disease phenotypes in SMA remains an important question. Here, we use whole-cell patch clamp technique to study primary motoneuron-astrocyte interactions in synaptic transmission. Primary embryonic spinal cord motoneurons from wild type (WT) or SMA Δ7

mutant mice were cultured alone or on a layer of either WT or mutant astrocytes for 12 days *in vitro*. AMPA- and kainate-receptors-mediated miniature excitatory postsynaptic currents (mEPSC)were recordedfromcultured motoneurons. In motoneuron cultures without astrocytes, we observed a ~63% reduction in mEPSC frequency recorded from mutant motoneurons, as compared with that from WT motoneurons. The result suggests an intrinsic defect in synaptic transmission in mutant spinal motoneurons. In WT motoneurons co-cultured with WT astrocytes, we recorded a ~3.5-fold increase in mEPSC frequency, in comparison with that in WT motoneurons alone, consistent with the emerging concept of astrocyte-induced synaptic enhancement. In WT motoneurons co-cultured with mutant astrocytes, there was a ~42% reduction of mEPSC frequency, as compared with that in WT motoneurons co-cultured with WT astrocytes. This result suggests that mutant astrocytes impair synaptic function in motoneurons. In mutant motoneurons co-cultured with either WT or mutant astrocytes, there was also a significant increase in mEPSC frequency in comparison with that of mutant motoneurons alone. The data suggests that astrocytes, whether they are WT or mutant, are capable of enhancing synaptic function even in mutant motoneurons. However, there was still a significant reduction in mEPSC frequency as compared with that in WT motoneurons co-cultured with WT astrocytes. Taking together, we have shown that SMA spinal motoneurons display an intrinsic deficit in synaptic function, and astrocytes from both WT and mutant mice can promote synaptic function in WT and mutant motoneurons, albeit to different degrees. However, mutations in astrocytes can impair the astrocyte-induced synaptic enhancement even in WT motoneuron culture. Our findings suggest that, in addition to the intrinsic defect in motoneurons, astrocytes are actively involved in the disease mechanisms. Therefore, it is essential to target not only motoneurons but also astrocytes for developing robust therapies in SMA.

Disclosures: **C. Zhou:** None. **Z. Feng:** None. **C. Ko:** None.

Nanosymposium

109. Astrocyte Action in CNS Disorders

Location: 144A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 109.12

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

Support: HFSP

ISF

BSF

Title: The role of astrocytes impairment in the etiology of Alzheimer's disease

Authors: *D. FRENKEL[1,2], D. FARFARA[1], V. LIFSHITZ[1], T. IRAM[1,2], S. AMRAM[1,2];
[1]Neurobiol, [2]Sagol Sch. of Neurosci., Tel Aviv Univ., Tel Aviv, Israel

Abstract: Astrocytes are the most abundant cells in the brain and play an important role in the homeostasis and maintenance of the brain. Furthermore, astrocytes play a key role in brain protection and in functional recovery from injuries. Dysfunction of astrocytes may promote neurodegeneration and, eventually, the retraction of neuronal synapses, which leads to cognitive deficits that are found in neurodegenerative diseases such as Alzheimer's disease (AD). The main pathology of AD is generally attributed to the increased production and accumulation of amyloid-beta (Abeta), in association with neurofibrillary tangle (NFT) formation. In AD patient brains, reactive astrocytes are integral components of neuritic plaques. Astrocytic activation seems to be particularly prominent around Abeta deposits both in the brain parenchyma and in the cerebrovasculature. We aim to investigate the role of astrocytes in the progression of AD. Astrocyte-endothelial cell (EC) interactions play a major role in the function of the neurovascular unit. Transforming growth factor- beta1 (TGF-beta1) expression levels positively correlate with the degree of cerebrovascular amyloid in Alzheimer's disease (AD) cases. Expression of TGF-beta1under astrocytes promoter in mice leads to an age-related deposition of amyloid, such as Abeta, around cerebral blood vessels. We demonstrated that TGF-beta1affects astrocyte and EC interaction leading to impairment in degradation and clearance of cerebrovascular amyloid in animal model. Hyperphosphorylation of tau correlates with NFT pathology in AD. We have discovered that intracellular hyperphosphorylation of tau in astrocytes leads to a reduction in the expression of glial fibrillary acidic protein (GFAP) and expression of neurotrophic factors such as vascular endothelial growth factor (VEGF), resulting in neurodegeneration in a new AD mouse model. Furthermore, those astrocytes showed impairment in their ability to engulf Abeta plaques leading to an increase in Abeta brain load and early cognitive impairment. Further research of the role of astrocyte impairment in AD may increase our understanding of the etiology of disease.

Disclosures: D. Frenkel: None. **D. Farfara:** None. **V. Lifshitz:** None. **T. Iram:** None. **S. Amram:** None.

Nanosymposium

11. Autism Synaptic and Cellular Mechanisms

Location: 144A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 11.01

Topic: C.06. Developmental Disorders

Support: HHMI grant 55007654

Title: Antagonism of metabotropic glutamate receptors reverses autistic behaviours caused by exacerbated mRNA translation initiation

Authors: *A. AGUILAR VALLES[1,2], E. MATTA CAMACHO[2], G. LING[2], K. NADER[2], J.-C. LACAILLE[1], N. SONENBERG[2];
[1]Univ. De Montreal, Montreal, QC, Canada; [2]McGill Univ., Montreal, QC, Canada

Abstract: Exacerbated mRNA translation during brain development is associated to several forms of autism spectrum disorders (ASD). We recently demonstrated that deletion of the eukaryotic Initiation Factor 4E-binding protein 2 (4E-BP2), a negative regulator of mRNA translation initiation, leads to an imbalance in excitatory-to-inhibitory neurotransmission and ASD-like behaviours. Antagonism of type I metabotropic glutamate receptors (mGluR, including mGluR1 and 5) has been successfully used to reverse ASD phenotypes in mouse models of Fragile X syndrome and in clinical trials. Importantly, these receptors activate mRNA translation initiation and elongation. We investigated the potential of treating autistic-like phenotypes by antagonists of mGluR1 (JNJ 16259685) and mGluR5 (Fenobam) in 4E-BP2 null mice. A single dose of mGluR1 (0.3 mg/kg) or mGluR5 (3 mg/kg) antagonists, which we established inconsequential in wild type mice, was sufficient to reverse the deficits in social exploration and exacerbated repetitive behaviours (marble burying and self-grooming) in 4E-BP2 knock outs. Exacerbated hippocampal long term depression (LTD), a form of synaptic plasticity that is translation dependent, was also normalized by either antagonist. Interestingly, although LTD becomes protein synthesis independent in 4E-BP2 mice (i.e. insensitive to elongation blocker anisomycin) this same treatment restored the levels of LTD back to those of wild type, suggesting that translational regulation downstream of initiation maybe amenable for regulation in 4E-BP2 null mice and can be a target of type I mGluR antagonists. We demonstrated that antagonism of type I mGluRs is a potential therapy that extends to autism models involving exacerbated mRNA translation initiation.

Disclosures: **A. Aguilar Valles:** None. **E. Matta Camacho:** None. **G. Ling:** None. **K. Nader:** None. **J. Lacaille:** None. **N. Sonenberg:** None.

Nanosymposium

11. Autism Synaptic and Cellular Mechanisms

Location: 144A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 11.02

Topic: C.06. Developmental Disorders

Support: SFARI 206683

Title: Repetitive behaviors in mice with specific deletion of Grip1/2 in Purkinje cells

Authors: *R. M. MEJIAS-ESTEVEZ[1], S.-L. CHIU[2], R. ROSE[3], M. HAN[3], R. L. HUGANIR[2], T. WANG[4];
[2]Neurosci., [3]Inst. of Genet. Med., [4]Pediatrics, [1]Johns Hopkins Univ., Baltimore, MD

Abstract: Cerebellar Purkinje cell (PC) loss and dysfunctions have been implicated in autism pathogenesis, although the molecular mechanisms are poorly understood. Glutamate receptor interacting protein 1 and 2 (GRIP1/2) are scaffolding proteins that regulate AMPA receptors recycling and synaptic transmission in the cerebellum. Previous studies from our laboratory have identified autism-associated mutations at Grip1/2 that result in more severe phenotype in autism patients. To study the role of GRIP1/2-mediated glutamate signaling at PCs in autism-associated phenotype, we have produced Purkinje cell-specific Grip1/2 double knockout (DKO) mice by crossing Grip2 conventional knockout (KO) and Grip1 conditional KO with Let-7 Cre mice. A pilot study showed that these DKO mice appeared to have normal number and morphology of Purkinje cells in cerebellum. We carried out a battery of behavior tests to study the phenotype of PC-specific Grip1/2 DKO male mice. Mutant mice showed normal levels of ambulatory activity, anxiety, sociability, and social novelty as compared to their wt littermates. Intriguingly, PC-specific Grip1/2 DKO mice presented a significant increase in cumulative time of stereotypic and repetitive grooming as compared to wt mice, and a mild deficit in motor and/or balance but not in motor learning in rotarod test. Increased stereotypic grooming in mice is a well-established, autism-specific behavior in autism mouse model. Our studies on PC-specific Grip1/2 KO mice suggest a crucial role of Grip1/2-mediated AMPA glutamate-signaling at PCs in stereotypic grooming behaviors in mice. Results from this study will provide valuable insights into the pathogenesis of stereotypic behaviors associated with autism.

Disclosures: R.M. Mejias-Estevez: None. **S. Chiu:** None. **R. Rose:** None. **M. Han:** None. **R.L. Huganir:** None. **T. Wang:** None.

Nanosymposium

11. Autism Synaptic and Cellular Mechanisms

Location: 144A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 11.03

Topic: C.06. Developmental Disorders

Title: Phenotypic effects of MeCP2 deletion in cholinergic neurons

Authors: *E. BALLINGER[1], C. SCHAAF[2], D. TALMAGE[3], H. Y. ZOGHBI[2,4,5], L. ROLE[3,6,7]; [1]Grad. Program in Neurosci., Stony Brook Neurosci., Stony Brook, NY; [2]Mol. and Human Genet., Baylor Col. of Med., Houston, TX; [3]Neurobio. and Behavior, Stony Brook Univ., Stony Brook, NY; [4]Howard Hughes Med. Inst., Chevy Chase, MD; [5]Jan and Dan Duncan Neurolog. Res. Inst. at Texas Children's Hosp., Houston, TX; [6]Ctr. for Nervous Syst. Disorders, Stony Brook, NY; [7]Neurosciences Inst., Stony Brook, NY

Abstract: Rett Syndrome (RTT) is an autism spectrum disorder that affects approximately 1 in 20,000 girls and is caused by mutations in the gene encoding methyl CpG binding protein 2 (*MeCP2*). The cholinergic system appears to be particularly important in RTT, as decreases in cholinergic markers have been correlated with increased clinical severity in patients with RTT. Schaaf and Zoghbi have developed a powerful transgenic mouse model, whereby *MeCP2* is selectively deleted in cholinergic neurons only, to facilitate study of the contribution of this cholinergic lesion to the overall phenotype of RTT. Interestingly, this model exhibits a selective deficit in recognition memory, a form of declarative memory that has been shown by lesion and electrophysiological studies to be dependent upon cholinergic signaling in the perirhinal cortex (PRH). This memory deficit may map onto the intellectual disability seen in patients with RTT, however, its molecular and electrophysiological underpinnings are unknown. We use optogenetics and in vivo electrophysiology to selectively activate cholinergic neurons in the Nucleus Basalis of Meynert (NBM), the cholinergic source nucleus that innervates the PRH, while simultaneously recording the effects of this selective activation in the PRH. We have demonstrated not only that NBM opto-stimulation modulates both the rate and variability of firing among PRH neurons, but that this modulation is impaired among selective cholinergic *MeCP2* knock-out mice. Additionally, we have found a decrease in expression of ChAT, the cholinergic synthetic enzyme, among selective *MeCP2* knock-out mice specifically in the NBM, while expression in other cholinergic nuclei is spared. This suggests that the recognition memory deficit seen among cholinergic *MeCP2* knock-out mice is mediated by deficient acetyl choline synthesis and signaling in the NBM-PRH circuit and that other cholinergic nuclei are robust to *MeCP2* deletion. These results may help guide the development of future targeted treatment strategies for patients with RTT.

Disclosures: E. Ballinger: None. **C. Schaaf:** None. **H.Y. Zoghbi:** None. **D. Talmage:** None. **L. Role:** None.

Nanosymposium

11. Autism Synaptic and Cellular Mechanisms

Location: 144A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 11.04

Topic: C.06. Developmental Disorders

Support: NIH Grant R01 DA017392

IDDRC Pilot Grant-NIH P30 HD071593

Title: Dysregulation of hippocampal inhibition in the CNTNAP2 knockout mouse

Authors: *S. JURGENSEN[1], P. E. CASTILLO[2];
[1]Dominick P. Purpura Neurosci. Dept., [2]Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., New York, NY

Abstract: Mutations in the gene encoding contactin-associated protein-like 2 (*CNTNAP2*) have been strongly associated with Autism Spectrum Disorders (ASDs). In particular, individuals carrying recessive *CNTNAP2* mutations tend to display language impairment and epilepsy phenotypes. *CNTNAP2*$^{-/-}$ mice represent a good model to study ASDs, as they reproduce the three core behavioral endophenotypes: social impairment, language deficits and repetitive behavior (Peñagarikano et al. 2011, Cell). In addition, *CNTNAP2*$^{-/-}$ mice show signs of asynchronous neuronal activity in the cortex and develop spontaneous seizures throughout adulthood, raising the possibility of an existing imbalance in synaptic activity. Despite its widespread expression in the brain, the only known function of the protein encoded by *CNTNAP2*, CASPR2, is to cluster potassium channels in the juxtaparanodes of myelinated axons in the PNS (Poliak et al., 1999, Neuron). Knockdown of *CNTNAP2* in cultured hippocampal neurons results in generalized decreases in multiple parameters of both excitatory and inhibitory synaptic transmission (Anderson et al., 2012, PNAS). However, the consequence of lifelong deletion of *CNTNAP2* to synaptic function in the intact brain remains unknown. In the present study, we have assessed basic synaptic transmission in acute slices of the hippocampus of *CNTNAP2*$^{-/-}$ mice. Strikingly, when compared to their wild-type littermates, *CNTNAP2*$^{-/-}$ mice show normal excitatory synaptic transmission at the Schaffer collateral to CA1 pyramidal neuron synapse, as assessed by normal input-output function, paired-pulse ratio, burst-mediated synaptic depression, NMDAR-AMPAR ratio, and mEPSC activity. In contrast, inhibition onto CA1 pyramidal cells was abnormal in *CNTNAP2*$^{-/-}$ mice as indicated by a rightward shift in the input-output function of evoked IPSCs, and an increase in the frequency, but not amplitude of spontaneous IPSCs. Remarkably, neither the frequency nor the amplitude of mIPSCs were

affected in *CNTNAP2*$^{-/-}$ mice, suggesting that the increase in spontaneous IPSCS could be due to hyperactive interneurons, a possibility that we are currently investigating. Changes in hippocampal inhibition could account for the epileptic phenotype developed by *CNTNAP2*$^{-/-}$ mice later in life, and potentially reflect a role for CASPR2 in clustering K^+ channels in interneurons. Overall, our findings suggest that *CNTNAP2* deletion affects hippocampal inhibition but not excitation. These findings provide further insights into the precise alterations in synaptic connectivity observed in ASDs, and could ultimately help elucidate the cellular and synaptic basis underlying these disorders.

Disclosures: S. Jurgensen: None. **P.E. Castillo:** None.

Nanosymposium

11. Autism Synaptic and Cellular Mechanisms

Location: 144A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 11.05

Topic: C.06. Developmental Disorders

Support: KO8 NINDS

Hearst Foundation Fellowship

Title: Identification of critical periods for treatment of autistic behavior in purkinje cell Tsc1 mice

Authors: *P. TSAI[1], Y.-X. CHU[2], B. BLUDEVICH[1], J. MOGAVERO[1], W. REGEHR[2], M. SAHIN[1];
[1]Neurol., Boston Children's Hosp., Boston, MA; [2]Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Background: Cerebellar Dysfunction has been implicated in the pathogenesis of autism spectrum disorders. By generating a mouse model where Tsc1 is deleted specifically in cerebellar Purkinje Cells, we have recently demonstrated that cerebellar dysfunction is sufficient to generate abnormal autistic behaviors and that early treatment with the mTOR inhibitor rapamycin can prevent the development of cerebellar pathology and autistic-like behavior. Objectives: Evaluate the benefits of later rapamycin treatment on autistic-like behaviors and to delineate critical periods of treatment for autistic-like behaviors. Methods: Using our Purkinje Cell Tsc1 mouse mutants, we have investigated the impact of rapamycin treatment initiated during adulthood on behavior, pathology, and electrophysiologic function to delineate the critical

periods of treatment of autistic behavior. Results: With rapamycin treatment starting at 6 weeks, we have demonstrated rescue of motor learning deficits and social behaviors - but not repetitive behaviors or cognitive inflexibility - in Purkinje Cell Tsc1 mutant mice. Rapamycin treatment at this time point also rescues pathologic and electrophysiologic deficits in these mice. Conclusion: These findings demonstrate that later treatment - even into adulthood - might offer benefit for social impairments. Furthermore, we demonstrate a critical period for treatment of social behaviors that differs from the critical period of rescue for motor learning, repetitive behaviors, and cognitive inflexibility, providing a platform for investigating the mechanisms underlying the critical periods for these behaviors and for further investigating the cerebellar contribution to autistic behavior.

Disclosures: P. Tsai: None. **Y. Chu:** None. **B. Bludevich:** None. **J. Mogavero:** None. **W. Regehr:** None. **M. Sahin:** 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.; Novartis Inc..

Nanosymposium

11. Autism Synaptic and Cellular Mechanisms

Location: 144A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 11.06

Topic: C.06. Developmental Disorders

Support: JSPS, Postdoctoral Fellowships for Research Abroad

International Rett Syndrome Foundation

NIH/NINDS 5R01NS057819-08

Howard Hughes Medical Institute

Title: Dissecting the roles of parvalbumin and somatostatin-positive interneurons in the pathogenesis of Rett syndrome

Authors: *A. ITO-ISHIDA[1,2], K. URE[1,2], H. Y. ZOGHBI[1,2,3];
[1]Mol. and Human Genet., Baylor Col. of Med., Houston, TX; [2]Jan and Dan Duncan Neurolog. Res. Inst. at Texas Children's Hosp., Houston, TX; [3]Howard Hughes Med. Inst., Houston, TX

Abstract: Rett syndrome (RTT) is an X-linked neurodevelopmental disorder caused by mutations of the gene encoding methyl-CpG-binding protein 2 (MeCP2). Typical symptoms of RTT are normal early development followed by regression, repetitive hand wringing, motor dysfunction, autistic features, and autonomic dysfunction. To understand the pathogenesis of RTT, our group has characterized multiple mouse lines that lack MeCP2 in specific subsets of neurons. Using this approach, it was discovered that mice lacking MeCP2 in inhibitory neurons recapitulate the majority of RTT symptoms (Chao et al., 2010, Nature). In addition, we have recently found that expressing MeCP2 selectively in inhibitory neurons was sufficient to rescue many RTT-related phenotypes (unpublished data). These results indicate that MeCP2 function in inhibitory neurons is critical for normal brain function and that its loss in this neuronal population is a key to RTT pathogenesis. Inhibitory neurons are classified into diverse subtypes. Although each subtype is known to have different physiological properties, how each subtype contributes to behavior in vivo remains elusive. Detailed analysis on the outcome of depleting MeCP2 from specific interneurons will provide insight into their contributions to specific behaviors. To examine how interneuron subtypes are involved in RTT pathogenesis, we focused on parvalbumin-positive (PV+) and somatostatin-positive (SOM+) neurons, each of which comprise about one third of cortical inhibitory neurons. Using Cre-loxP technology, we generated mice lacking MeCP2 specifically in PV+ cells (PV-conditional knock outs: PV-CKOs) and SOM+ cells (SOM-CKOs). The mice were characterized by multiple behavioral assays, and the results were compared between each CKO and its littermate control groups. We found that PV-CKOs have stronger phenotypes in multiple behavior assays, whereas SOM-CKOs have less but distinct phenotypes that were not present in PV-CKOs. Phenotypes specific to PV-CKOs were motor dysfunction, rigidity, reduced acoustic startle response, and impaired cued memory. Phenotypes specific to SOM-CKOs were epileptic seizure and an increase in compulsive behavior that was measured by hole board assay. Interestingly, both PV and SOM-CKOs had reduced life span. When combined together, these behavioral phenotypes observed in PV and SOM-CKOs covered the majority of phenotypes observed in mice lacking MeCP2 in all inhibitory neurons. Our findings imply differential roles of PV+ and SOM+ cells in the pathogenesis of RTT. The data will provide an important framework for functional studies that will further characterize these inhibitory neuronal subtypes.

Disclosures: **A. Ito-Ishida:** None. **K. Ure:** None. **H.Y. Zoghbi:** None.

Nanosymposium

11. Autism Synaptic and Cellular Mechanisms

Location: 144A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 11.07

Topic: C.06. Developmental Disorders

Support: MRC

Title: The autism and schizophrenia associated gene CYFIP1 is critical for the maintenance of dendritic complexity and the stability of mature spines

Authors: *E. C. DAVENPORT, M. PATHANIA, J. MUIR, D. F. SHEEHAN, G. LÓPEZ-DOMÉNECH, J. T. KITTLER;
Neuroscience, Physiol. and Pharmacol., Univ. Col. London, London, United Kingdom

Abstract: Copy number variation (CNV) at the 15q11.2 region of the genome has been identified as a significant risk locus for neuropsychiatric disorders such as autism spectrum disorder (ASD), schizophrenia (SCZ), bipolar disorder and intellectual disability (ID). Investigating the cellular function of genes within this region is important to understand how neuropsychiatric disorders develop. *Cyfip1* is a gene within this region that has been implicated in the pathogenesis of ASD and SCZ. Therefore, understanding how CNV or mutations in *Cyfip1* impair nervous system development, function and connectivity resulting in these disorders is an important goal. Precise control of actin dynamics is critical for the correct development and maintenance of neuronal networks, dendritic arbours and actin-rich spines. *Cyfip1* encodes an essential component of the WAVE regulatory complex (WRC), a complex vital for actin regulation. CYFIP1 maintains the WRC in an inhibited state until the small GTPase Rac1, once activated, binds CYFIP1. This interaction results in CYFIP1 dissociating from the WRC allowing the complex to trigger actin assembly. Here we investigate how *Cyfip1* expression level and mutations in *Cyfip1* impact its actin regulatory function and its role in the regulation of neuronal morphogenesis and synaptic maintenance using mouse genetics, overexpression and imaging techniques. We show CYFIP1 is highly enriched at synapses and its overexpression leads to increased dendritic complexity. Neurons derived from *Cyfip1*$^{+/-}$ animals on the other hand possess reduced dendritic complexity, increased mobile F-actin and enhanced GluA2-containing AMPA receptor mobility at synapses. Interestingly, both *Cyfip1* overexpression and haploinsufficiency increased immature spine number. Thus, CYFIP1 dysregulation leads to pathological changes in CNS maturation and neuronal connectivity. Using similar approaches we investigate how disease associated mutations in *Cyfip1* influence its function. Taken together our findings provide new insights into how genetic alterations in *Cyfip1* may contribute to the development of the neurological symptoms seen in ASD, SCZ and ID.

Disclosures: E.C. Davenport: None. **M. Pathania:** None. **J. Muir:** None. **D.F. Sheehan:** None. **G. López-Doménech:** None. **J.T. Kittler:** None.

Nanosymposium

11. Autism Synaptic and Cellular Mechanisms

Location: 144A

Time: Saturday, November 15, 2014, 1:00 PM - 3:00 PM

Presentation Number: 11.08

Topic: C.06. Developmental Disorders

Support: NIH Grant R01-NS065027

IRSF Postdoctoral Fellowship IRSF-2824

Title: Excitatory CA3->CA1 synapses are stronger in Mecp2 knockout mice and saturate long-term potentiation

Authors: *W. LI, L. POZZO-MILLER;
Neurobio., The Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Unbalanced excitatory and inhibitory synaptic function leading to neuronal network instability has emerged as a common feature in disorders associated with intellectual disabilities, including Rett syndrome (RTT). Using the Mecp2 knockout mouse model of RTT, we found that excitatory synapses onto CA1 pyramidal neurons in hippocampal slices from symptomatic mice have all the hallmarks of "already potentiated" synapses compared to WT neurons: miniature EPSCs in TTX are larger, the slope of input-output curves of evoked EPSCs is steeper, the ratio of AMPAR/NMDAR EPSCs is larger, and membrane currents evoked by bath-applied AMPA are larger. In addition to those biophysical hallmarks of potentiated synapses, the expression levels of GluA1 subunits are higher in Mecp2 knockout mice, as determined by Western immunoblots and semi-quantitative confocal immunohistochemistry. Consistent with this evidence of stronger synapses, either theta-burst stimulation (TBS) of CA3 afferent fibers, or low-frequency afferent stimulation during postsynaptic depolarization failed to potentiate whole-cell EPSCs (and change the A/N ratio), field EPSPs, and the amplitude and spatio-temporal spread of voltage-sensitive dye signals in CA1 of Mecp2 knockout mice. Furthermore, dendritic spines of Mecp2 knockout CA1 pyramidal neurons had larger volumes than spines in wildtype neurons, and did not show the characteristic increase in volume after LTP induction, as determined by 3D reconstructions from confocal z-stacks of biocytin-filled cells during whole-cell recordings. We next examined trafficking of GluA1-containing AMPARs and found that cultured Mecp2 knockout hippocampal neurons had a higher surface-to-total GluA1 ratio than wildtype cells, and failed to show the increase in surface GluA1 after glycine-induced LTP. Similarly, time-lapse imaging of SEP-GluA1 demonstrated that GluA1 intensity did not increase in naïve Mecp2 knockout neurons after glycine-induced chemical LTP, like it did in wildtype

cells, indicating impaired surface delivery of pH-sensitive GluA1 into synapses of Mecp2 knockout neurons. We are currently investigating the molecular bases of impaired AMPAR trafficking into and out of hippocampal synapses during activity-dependent plasticity. Collectively, our findings provide molecular, cellular, and network mechanisms of impaired synaptic transmission and plasticity in Mecp2 knockout mice, which will aid to the rational design and pre-clinical evaluation of novel therapies for RTT.

Disclosures: W. Li: None. **L. Pozzo-Miller:** None.

Nanosymposium

110. Alzheimer's Disease: Genetics and Biology I

Location: 152A

Time: Sunday, November 16, 2014, 8:00 AM - 11:30 AM

Presentation Number: 110.01

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

Support: NIH Grant P01-AGO30128

NIH Grant R01-AG045775

University of Kentucky Bucks for Brains Program

Title: Translating CD33 genetics to an Alzheimer’s disease prophylactic

Authors: M. MALIK, J. SIMPSON, *S. ESTUS;
Sanders-Brown Ctr. Aging, LEXINGTON, KY

Abstract: Recent genome-wide association studies have identified the single nucleotide polymorphism (SNP) rs3865444, located 372 base pairs upstream of CD33, as a modulator of Alzheimer’s disease (AD) susceptibility. CD33 is a sialic-acid binding inhibitory receptor, postulated to have an immunosuppressive effect on microglia in brain, but the function of rs3865444 in AD has been unclear. Here, we illustrate the mechanism of rs3865444 action on CD33 splicing and expression in human brain. Our long-term goal is to develop pharmacologic agents that mimic the effects of protective SNPs to combat AD onset. We begin by identifying CD33 isoforms expressed in human brain, finding a predominant form that lacks exon 2 (D2-CD33) and a form that retains intron 1 (R1-CD33). Using qPCR, we quantify total CD33, D2-CD33, and R1-CD33 from cDNA prepared from 30 AD and 30 non-AD brains. We find a significant association between exon 2 skipping and rs386544 genotype, indicating that the AD-protective allele of the SNP promotes skipping of exon 2 and production of non-functional

CD33. We identify a co-inherited exon 2 SNP, rs12459419, as the functional SNP that modulates exon 2 splicing. We find that rs3865444 also associates with increased retention of intron 1, leading to production of a prematurely truncated CD33 protein. Thus, we conclude that the minor allele of rs3865444 functions to decrease AD risk by inhibiting CD33 function, thereby enabling microglial mobilization against amyloid. We propose that an antibody treatment that clears surface CD33 will be prophylactic against AD; preliminary results are presented here.

Disclosures: **M. Malik:** None. **J. Simpson:** None. **S. Estus:** None.

Nanosymposium

110. Alzheimer's Disease: Genetics and Biology I

Location: 152A

Time: Sunday, November 16, 2014, 8:00 AM - 11:30 AM

Presentation Number: 110.02

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

Support: CIHR Grant CCI-117952

Title: A Novel SNP in Tmp21 in the Patients with Alzheimer’s disease

Authors: ***X. ZHANG**[1], K. XIA[2], Y. WU[1], C. FANG[1], K. BROMLEY-BRITS[1], W. SONG[1]; [1]Dept. of Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada; [2]The State Key Lab. of Med. Genet., Central South Univ., Changsha, China

Abstract: TMP21, a type I transmembrane protein of the p24 protein family is a trafficking protein. Recent studies suggest that TMP21 is a selective modulator of γ-secretase and its dysregulation may play a pivotal role in Alzheimer’s disease (AD) pathogenesis. It has been reported that TMP21 modulates γ-secretase activity and APP trafficking, leading to the increase of Aβ generation. *Tmp21* is located on Chr14q24.3, the region highlighted by AD linkage studies. However, the genetic association between *Tmp21* and AD remains elusive. In this study, we first identified that a novel T>C Single-Nucletide Polymorphism (SNP) located in intron 4 of *Tmp21* and is associated with AD patients by screening 261 AD patients and 236 controls. The SNP did not affect the splicing site recognition, but it significantly increased TMP21expression at both mRNA and protein levels. Furthermore, we found that this SNP significantly increased the splicing efficiency of *Tmp21* pre-mRNA, leading to the elevation of mature mRNA. However, the stability of *Tmp21* pre-mRNA and transcription activity of *Tmp21* was not affected. Taken together, our study not only identified an AD-

associated *Tmp21* SNP, but also indicated that dysregulation of TMP21 may contribute to AD pathogenesis and that TMP21 may be a potential target for AD treatment.

Disclosures: **X. Zhang:** None. **K. Xia:** None. **Y. Wu:** None. **C. Fang:** None. **K. Bromley-Brits:** None. **W. Song:** None.

Nanosymposium

110. Alzheimer's Disease: Genetics and Biology I

Location: 152A

Time: Sunday, November 16, 2014, 8:00 AM - 11:30 AM

Presentation Number: 110.03

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

Support: NIRG-12-242187

NIH/NIGMS U54GM104942

Title: The role of the tripartite glutamatergic synapse in the pathophysiology of Alzheimer's disease

Authors: *M. N. REED[1], H. HUNSBERGER[2], D. WEITZNER[2], C. RUDY[2];
[1]Dept. of Psychology, [2]West Virginia Univ., Morgantown, WV

Abstract: Alzheimer's disease (AD) is characterized by accumulation of beta-amyloid (Aβ) and tau, synapse loss, neural network dysfunction, and eventually neuron loss. A growing body of evidence suggests perturbations in the glutamatergic tripartite synapse, comprised of a presynaptic terminal, a postsynaptic spine, and an astrocytic process, may underlie the pathogenic mechanisms of AD. Here, we describe novel alterations in glutamatergic tripartite synapse that occur early in the disease process and may represent therapeutic targets. To examine glutamate regulation in vivo, we used an amperometry coupled to ceramic-based microelectrode arrays (MEAs), which allows for measurement of tonic glutamate levels, potassium-evoked glutamate release, and glutamate clearance from the synapse. Glutamate regulation was measured separately in the dentate gyrus (DG), CA3 and CA1 regions of the hippocampus and correlated with memory performance in hippocampal-dependent memory tasks. To control for the overexpression of human tau, we also examined glutamate regulation in mice expressing wild-type human tau (TauWT) at levels equivalent to that of mice expressing P301L tau (TauP301L). We show that TauP301L mice exhibited a 7-fold increase in glutamate release in the CA3 region of the hippocampus, and spatial reference memory errors correlated with

glutamate release in the CA3. This increase in glutamate release was associated with a 65% increase in presynaptic vesicular glutamate transporter (VGLUT) expression. We next examined the ability of riluzole, an FDA-approved disease-modifying drug for amyotrophic lateral sclerosis that effectively regulates glutamate levels, to attenuate memory deficits and glutamate release in TauP301L mice. Preliminary results suggest riluzole effectively attenuates pathology in TauP301L mice. These data suggest a possible novel mechanism, increased presynaptic glutamate release, by which tau may mediate synaptic alterations. Findings of increased CA3 glutamate release in our mouse model corroborate findings of CA3 hyperexcitability in memory-impaired aged humans and patients with mild cognitive impairment (MCI). Our findings also suggest riluzole may serve as an effective meditator of tau-related pathology.

Disclosures: **M.N. Reed:** None. **H. Hunsberger:** None. **D. Weitzner:** None. **C. Rudy:** None.

Nanosymposium

110. Alzheimer's Disease: Genetics and Biology I

Location: 152A

Time: Sunday, November 16, 2014, 8:00 AM - 11:30 AM

Presentation Number: 110.04

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

Support: Research to Prevent Blindness (RPB)

COBRE III NIH/NIGMS Grant P30-GM103340

Memory Impairments and Neurological Disorders (MIND) Institute and the University of California, Irvine Alzheimer's Disease Research Center (UCI-ADRC; NIA P50 AG16573)

Louisiana Biotechnology Research Network (LBRN)

NEI EY006311

NIA AG18031

NIA AG038834

Title: Circular RNA (circRNA) ciRS-7 mediates microRNA-7 (miRNA-7) trafficking in Alzheimer's disease (AD)

Authors: *W. J. LUKIW[1], Y. ZHAO[1], S. BHATTACHARJEE[1], M. PERCY[2], A. POGUE[3], P. DUA[4];
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Abstract: Circular RNAs (circRNAs) are a naturally occurring family of small noncoding RNAs (sncRNAs) highly represented in the eukaryotic transcriptome. Recently characterized, traditional methods of RNA detection and analysis requiring a free 5′ or 3′ ribonucleotide terminus may have significantly underestimated circRNA abundance and significance. Intrinsically resistant to exonucleolytic RNA decay, circRNAs appear to be enriched in mammalian brain tissues. Interestingly, specific sncRNAs such as the evolutionary ancient homo sapien microRNA-7 (hsa miRNA-7; chr 9q21.32; ~23 nt; http://www.mirbase.org/cgi-bin/mirnaentry.pl?acc= MI00 00263; a known, important post-transcriptional regulator of phagocytosis), are not only very abundant in the human CNS, but are also associated with a circRNA for miRNA-7 (ciRS-7) in the same tissues. ciRS-7 contains about ~70 tandem anti-miRNA-7 sequences; ciRS-7 (~1400 nt) thereby acts as a kind of endogenous, competing, anti-complementary miRNA "sponge" to adsorb, and hence quench, normal miRNA-7 function. Using miRNA arrays, enhanced Northern blot hybridization and the circularity-sensitive probe RNaseR we here provide initial evidence of a mis-regulated ciRS-7-miRNA-7 system in sporadic Alzheimer's disease (AD). Deficits in ciRS-7, and ciRS-7 "sponging activities" might be expected to increase ambient miRNA-7 levels in AD-affected brain cells, as is observed, to ultimately contribute to the down-regulation of selective miRNA-7-sensitive messenger RNA (mRNA) targets. The presence of up-regulated miRNA-7, due to a deficiency in ciRS-7 "sponging" effects, was shown to down-regulate AD-relevant targets, such as, for example, the ubiquitin protein ligase A (UBE2A; miRNA-7-UBE2A mRNA energy of association, E_A = −22.86 kcal/mol). UBE2A, an autophagic, phagocytic protein essential in the proteasome-mediated clearance of amyloid peptides is depleted in AD brain. Such circRNA-miRNA-mRNA regulatory systems appear to represent another important layer of epigenetic control over gene expression in the CNS. Indeed, our ideas on sncRNAs in the CNS continue to evolve, and technological advancement, refinement and recent discoveries continue to challenge the basic doctrines of nucleic acid biochemistry and evolutionary neurobiology in both health and disease.

Disclosures: W.J. Lukiw: None. **Y. Zhao:** None. **S. Bhattacharjee:** None. **M. Percy:** None. **A. Pogue:** None. **P. Dua:** None.

Nanosymposium

110. Alzheimer's Disease: Genetics and Biology I

Location: 152A

Time: Sunday, November 16, 2014, 8:00 AM - 11:30 AM

Presentation Number: 110.05

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

Support: the Fundamental Research Funds for the Central Universities of China 21614331

NIH grant R01 HG006465

Title: Whole-exome sequencing identifies the genetic causes in a family with idiopathic progressive cognitive decline

Authors: *L. SHI[1,2,3], K. WANG[4,5];
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Abstract: Background: In developing countries, for rare neurological diseases with known genetic basis, a significant number of cases remain undiagnosed or misdiagnosed due to the limited diagnostic experience and the lack of well-trained medical geneticists. We have encountered a case where two children from a family quartet have an idiopathic progressive cognitive decline, and were suspected to be undiagnosed untypical neurodevelopmental disease, but the disease cause remains unknown for a number of years. We attempted to solve the genetic cause by exome sequencing. Methods: The patients' initial clinical assessment included history and physical examination, cranial MRI, and nerve conduction studies. We performed whole-exome sequencing on two affected children from the family on the Illumina HiSeq2500 platform with Agilent exome capture, followed by variant annotation and selection of rare, shared variants that fit a recessive model of inheritance by ANNOVAR software. We used Sanger sequencing to confirm the candidate gene and variants on all family members. To validate the functional impacts of the candidate variants, we used enzymology test to confirm the biochemical activity of several enzymes. Results: We identified NAGLU as the most likely candidate gene for the disease, and validated that both probands are compound heterozygote for two known disease causal mutations by Sanger sequencing. Biochemical tests confirmed the decreased activity of NAGLU, confirming the genetic diagnosis of Sanfilippo syndrome IIIB. Conclusions: Our study represents an example of using whole-exome sequencing identify the genetic causes of idiopathic disorders in clinical settings. Exome sequencing provides a powerful tool in aiding diagnosis of "mild" or "atypical" neurometabolic disorders.

Disclosures: **L. Shi:** None. **K. Wang:** None.

Nanosymposium

110. Alzheimer's Disease: Genetics and Biology I

Location: 152A

Time: Sunday, November 16, 2014, 8:00 AM - 11:30 AM

Presentation Number: 110.06

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

Title: A genome-wide siRNA screen to identify novel targets and pathways regulating apoE secretion in human astrocytes

Authors: *S. HASSON[1], Z. WENZEL[2], R. YANG[1], M. LIU[3], L. LANYON[2], R. SINGLETON[2], C. STEPPAN[2], M. KUHN[2], S. XI[1], N. BODYCOMBE[1], M. GLICKSMAN[3], J. CONCANNON[3], K. BALES[1], G. RAMASWAMY[1];
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Abstract: Apolipoprotein E (apoE) is a key lipid transport protein in brain and plasma. In the brain, astrocytic apoE is primarily responsible for the delivery of lipids to neurons via receptors on the cell surface. Humans have three isoforms of the protein: apoE2, apoE3 and apoE4. Of these three isoforms, apoE4 is significantly linked to late onset and sporadic forms of Alzheimer’s disease (LOAD) while apoE2 is associated with a reduced risk of LOAD. To date, the apoE4 genotype is the most significant risk factor for the development of LOAD . Given its critical role in neuronal repair, synaptogenesis, and clearance of toxic Aß fragments from brain, apoE has been suggested to play an important functional role in AD pathogenesis. Therefore, modulating brain apoE levels could represent a promising therapeutic strategy for treating AD. However, the mechanisms that regulate apoE expression and secretion in astrocytes are poorly understood. We used a genome-wide siRNA screen to investigate targets and pathways that regulate apoE secretion in a human astrocytoma cell line. Our high-throughput assay for measuring apoE secretion used a robust alphaLISA endpoint capable of detecting both enhancing and inhibitory knockdown phenotypes in the well-characterized astrocytoma model. After selecting 300 promising candidates from the primary screen for further study, 51 genes were reconfirmed in subsequent studies with at least 2 out of 4 distinct siRNA reagents for a given gene recapitulating activity. We then employed an array of secondary assays including alternative siRNA chemistries, c911 control reagents, and RNAseq to rule-out possible off-target effects that are known to confound data from siRNA screens. Pathway analysis of the 51 reconfirmed candidate genes has illuminated established links with lipid transport processes in addition to previously unexplored biology. Our novel approach to map the genome-wide

landscape of apoE regulation has the potential to reveal important and unappreciated mechanisms associated with the regulation of astrocytic apoE secretion.

Disclosures: **S. Hasson:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **Z. Wenzel:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **R. Yang:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **M. Liu:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Pfizer, Inc. **L. Lanyon:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **R. Singleton:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **C. Steppan:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **M. Kuhn:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **S. Xi:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **N. Bodycombe:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **M. Glicksman:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Pfizer, Inc. **J. Concannon:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Pfizer, Inc. **K. Bales:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **G. Ramaswamy:** A. Employment/Salary (full or part-time):; Pfizer, Inc..

Nanosymposium

110. Alzheimer's Disease: Genetics and Biology I

Location: 152A

Time: Sunday, November 16, 2014, 8:00 AM - 11:30 AM

Presentation Number: 110.07

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

Title: Identification of Alzheimer’s disease protective genetic variants in non-demented elderly individuals with APOE ε4/ε4 genotype

Authors: ***A. N'SONGO**[1,2], M. M. CARRASQUILLO[3], Y. ASMANN[4], S. BAHETI[6], G. ZHANG[4], T. NGUYEN[3], R. J. CASELLI[8], N. R. GRAFF-RADFORD[5], R. C. PETERSEN[7], G. BU[3], N. ERTEKIN-TANER[5,3];
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Abstract: Background: Alzheimer’s disease (AD) is the most common type of dementia. AD can be divided into 2 different categories: early onset AD (EOAD) which accounts for <1% of all AD cases and is known to be caused by dominant mutations; and late onset AD (LOAD) resulting from complex interactions between environmental and genetic risk factors.

Apolipoprotein (*APOE*) isoform ε4 is the most well established and strongest genetic risk factor for LOAD with an increased risk of ~4 fold for heterozygous carriers of this allele and up to ~30 fold for homozygote carriers. However, it does not account for all LOAD cases and is not alone sufficient to cause the disease. Our group has access to a unique longitudinally followed cohort of 24 *APOE* ε4/ε4 non-demented individuals aged ≥ 75 years old at last evaluation. The goal of this study is to identify genetic variants associated with protection from LOAD in those cognitively intact *APOE* ε4/ε4 elderly carriers. **Method:** Whole exome sequencing was performed on genomic DNA samples using the Sure Select V4 + UTR Exome Capture kit on the Illumina HiSeq platform. After quality control analysis, coding variants were prioritized by association p-value for Fisher's exact test against Exome Variant Server Caucasian controls. Variants with a p-value > 0.05 were excluded. The variants which were selected for follow-up genotyping by Sequenom in an AD case-control cohort, met the following criteria: 1) Variants significantly enriched in our cohort (p-values < $3.3x10^{-5}$ after Bonferroni correction), as well as any other variants in those same genes with a SIFT or Polyphen prediction score of possibly damaging or damaging, 2) Variants with a suggestive enrichment in our cohort (p-values < $1x10^{-3}$) and a SIFT or Polyphen prediction score of possibly damaging or damaging, 3) Variants within candidate AD risk genes. **Results:** After statistical analysis, several variants showed a significant association for enrichment in *APOE* ε4/ε4 non-demented elderly cohort compared to the general population. **Conclusion:** Whole exome sequencing of elderly, non-demented subjects with *APOE* ε4/ε4 genotypes can identify coding variants that may be protective against AD. The study of the functional effect of those variants would allow us to have a better understanding of the molecular mechanism of AD and to identify possible biomarkers and therapeutic targets.

Disclosures: **A. N'Songo:** None. **M.M. Carrasquillo:** None. **Y. Asmann:** None. **S. Baheti:** None. **G. Zhang:** None. **T. Nguyen:** None. **R.J. Caselli:** None. **N.R. Graff-Radford:** None. **R.C. Petersen:** None. **G. Bu:** None. **N. Ertekin-Taner:** None.

Nanosymposium

110. Alzheimer's Disease: Genetics and Biology I

Location: 152A

Time: Sunday, November 16, 2014, 8:00 AM - 11:30 AM

Presentation Number: 110.08

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

Support: Health Research Council of the Academy of Finland, EVO grant 5772708 of Kuopio University Hospital

the Strategic Funding of the University on Eastern Finland (UEF-Brain)

Sigrid Juselius Foundation

FP7, Grant Agreement no 601055, VPH Dementia Research Enabled by IT VPH-DARE@IT

Tampere University Hospital Medical Fund VTR (EVO) grant 9N035 and X51001

Title: Effects of Alzheimer's disease-associated risk loci on cerebrospinal fluid biomarkers and disease progression: A polygenic risk score approach

Authors: H. MARTISKAINEN[1], S. HELISALMI[1], J. VISWANATHAN[1], M. KURKI[2], A. HALL[1], S.-K. HERUKKA[1], T. SARAJÄRVI[1], T. NATUNEN[1], *K. M. KURKINEN[3], J. HUOVINEN[1], A. M. REMES[1], A. M. KOIVISTO[1], K. M. MATTILA[4], T. LEHTIMÄKI[4], M. LAITINEN[1], P. MÄKINEN[1], V. LEINONEN[2], A. HAAPASALO[1], H. SOININEN[1], M. HILTUNEN[1];
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Abstract: Several new risk loci for Alzheimer's disease (AD) have been recently identified in large-scale genome wide association studies. However, little is known about the role of these loci in the AD pathogenesis. We investigated the association of 12 newly identified risk loci with AD in a clinical cohort consisting of 890 AD patients and 701 age-matched healthy controls from Kuopio, Oulu, and Tampere in Finland. DNA was extracted from peripheral blood and the selected single nucleotide polymorphisms were genotyped using Sequenom iPlex platform. Association of the risk loci with AD-related cerebrospinal fluid (CSF) biomarkers was studied among 222 AD patients. To assess the combined risk effect of top 22 AD risk loci, we used a polygenic risk score approach by calculating risk score for each individual by summing log-transformed odds ratios reported in meta-analyses, weighted by the number of alternative alleles carried by the individual. Within the clinical cohort, the effects of polygenic risk score was assessed in relation to CSF biomarker levels. We also included a neuropathological cohort consisting of post-mortem temporal cortex samples from 59 individuals with well-defined AD-related neurofibrillary changes, and assessed the effects of polygenic risk score in relation to neurofibrillary pathology and α-, β-, and γ-secretase activities and soluble amyloid-β (Aβ) levels measured from the temporal cortex samples. We observed a protective effect among the minor allele carriers of SLC24A4 rs10498633 and EPHA1 rs11771145, respectively, in our clinical case-control cohort. Interestingly, these two loci also associated with increased CSF Aβ42 levels. FERMT2 rs17125944 associated with decreased levels of total and phosphorylated tau in the CSF. Polygenic risk scores associated with CSF Aβ42 levels in the clinical cohort, and with soluble Aβ42 levels and γ-secretase activity in the neuropathological cohort. To conclude, AD risk genes polygenically contribute to Aβ pathology in the CSF and in the temporal cortex, and this effect is potentially associated with increased γ-secretase activity. Furthermore, we report for

the first time an association between risk loci in genes SLC24A4, EPHA1 and FERMT2 and AD-related CSF biomarkers.

Disclosures: **H. Martiskainen:** None. **S. Helisalmi:** None. **J. Viswanathan:** None. **M. Kurki:** None. **A. Hall:** None. **S. Herukka:** None. **T. Sarajärvi:** None. **T. Natunen:** None. **K.M. Kurkinen:** None. **J. Huovinen:** None. **A.M. Remes:** None. **A.M. Koivisto:** None. **K.M. Mattila:** None. **T. Lehtimäki:** None. **M. Laitinen:** None. **P. Mäkinen:** None. **V. Leinonen:** None. **A. Haapasalo:** None. **H. Soininen:** None. **M. Hiltunen:** None.

Nanosymposium

110. Alzheimer's Disease: Genetics and Biology I

Location: 152A

Time: Sunday, November 16, 2014, 8:00 AM - 11:30 AM

Presentation Number: 110.09

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

Support: P01-AG030128

Title: Exploring a genetic link between Alzheimer's disease and Type 2 Diabetes Mellitus: The AD-associated SNP in CELF1 is co-inherited with the T2DM-associated SNP in MADD

Authors: *J. VASQUEZ[1], S. ESTUS[2], J. SIMPSON[2];
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Abstract: Genome wide association studies (GWAS) have recently identified a chromosome 11 locus that contains single nucleotide polymorphisms (SNPs) associated with Alzheimer's disease (AD) risk. Genes within this locus include *CELF1*, which encodes a RNA-binding protein implicated in post-transcriptional events within the brain and *MADD,* which encodes an adapter protein to mediate TNFR1 signaling. Separate GWAS have identified a SNP within *MADD* (rs10501320) as significant (p=10x10^{-88}) for fasting insulin and glucose levels, traits associated with type 2 diabetes mellitus (T2DM); the AD and T2DM SNPs are co-inherited (r^2=0.51). Since T2DM is a risk factor for AD, we hypothesize that these SNPs are associated with function of CELF1 or MADD to influence both AD and T2DM risk. Since the Exome Variant Server indicates that there are no amino-acid changing SNPs within *CELF1* or *MADD* that are co-inherited with the AD or T2DM SNPs, we hypothesize that the SNPs are associated with altered *CELF1* or *MADD* gene expression. On the BioPortal SNP website (app3.titan.uio.no/biotools/tool1) evaluating eQTLs in transformed lymphocytes, both the AD SNP rs10838725 and the T2DM SNP rs10501320 are associated with a MADD isoform

(p=4.1x10^{-5}), but weakly or not associated with CELF1 expression (p values range from 0.008-0.454). To evaluate this hypothesis in brain, we are currently genotyping the AD and T2DM-associated SNPs in 60 human brain RNA samples and quantifying *CELF1* and *MADD* expression by qPCR. Hence, we will pursue the extent that CELF1 or MADD expression may modulate AD risk and provide a genetic link between AD and T2DM.

Disclosures: J. Vasquez: None. **S. Estus:** None. **J. Simpson:** None.

Nanosymposium

110. Alzheimer's Disease: Genetics and Biology I

Location: 152A

Time: Sunday, November 16, 2014, 8:00 AM - 11:30 AM

Presentation Number: 110.10

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

Support: NIH AG030205

Title: Homeostatic role of nitric oxide signaling maintains synaptic plasticity expression in Alzheimer's disease mice

Authors: S. CHAKROBORTY[1], C. BRIGGS[1], J. KIM[3], C. SCHNEIDER[3], A. WEST[1], *G. E. STUTZMANN[2];
[1]Neurosci., [2]Rosalind Franklin Univ. /Chicago Med. Sch., NORTH CHICAGO, IL; [3]Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA

Abstract: Alterations in synaptic plasticity contribute to cognitive deficits in neurological disorders such as Alzheimer's disease (AD). To ensure proper information encoding, neuronal systems recruit numerous regulatory mechanisms. Of these, calcium signaling is fundamental, and can activate second messengers such as nitric oxide (NO) which modulate synaptic plasticity. Across AD mouse models, calcium release is markedly increased in CA1 neurons, and NO synthase levels, and likely NO, are increased. Yet, Ca2+-dependent synaptic transmission and plasticity appear normal, despite an underlying reversion to synaptic depression with ryanodine receptor (RyR) block. This suggests homeostatic mechanisms may maintain the appearance of normal synaptic plasticity early in AD pathogenesis. Our goal was to investigate the interaction between increased RyR-calcium release and NO modulation of synaptic plasticity, and identify a possible compensative role of NO in AD brains. Since NO increases RyR activity, blocking NO synthesis with L-NAME could decrease the aberrant calcium responses in AD. To explore this negative feedback loop, we combined 2-photon Ca2+ imaging with patch clamp

electrophysiology in acute hippocampal brain slices from presymptomatic 3xTg-AD and NonTg mice. We found that synaptically-evoked Ca2+ responses in 3xTg-AD CA1 neurons were 3-fold higher in dendrites and spines relative to NonTg neurons. Blocking NO decreased the evoked Ca2+ response in 3xTg-AD neurons (no effect in NonTg neurons), and resulted in significantly different synaptic plasticity patterns. Under control conditions, synaptic depression was increased in 3xTg-AD neurons immediately after an LTD-inducing stimulus (0-3 minutes post LFS), while short-term depression (STD; 15-20 minutes post LFS) was similar to NonTg. Inhibiting NO resulted in greater synaptic depression after LFS as well as enhanced STD in 3xTg-AD neurons, while this treatment abolished STD in NonTg neurons. Our extracellular field potential studies show similar responses - blocking NO blocked the expression of short-term and long-term depression in NonTg, whereas in AD mice, short-term and long-term synaptic depression was significantly increased. Thus, NO may counteract the aberrant RyR-calcium mediated predisposition towards synaptic depression in AD mice. NO generation, mediated through calcium, can play an important negative feedback role in preventing maladaptive synaptic depression in AD. These findings support the hypothesis that homeostatic mechanisms are present at presymptomatic disease stages, and preserve synaptic function before behavioral symptoms emerge.

Disclosures: **S. Chakroborty:** None. **C. Briggs:** None. **J. Kim:** None. **A. West:** None. **C. Schneider:** None. **G.E. Stutzmann:** None.

Nanosymposium

110. Alzheimer's Disease: Genetics and Biology I

Location: 152A

Time: Sunday, November 16, 2014, 8:00 AM - 11:30 AM

Presentation Number: 110.11

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

Support: NIH NS081706

NIH NS34389

NIH ADRC AG005138

EMBO

SNF

Title: nELAVL binding shifts to non-coding Y RNAs during Alzheimer's Disease progression and stress

Authors: *C. SCHECKEL[1], E. DRAPEAU[2], M. FRIAS[1], J. D. BUXBAUM[2], R. B. DARNELL[1];
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Abstract: Neuronal ELAV-like (nELAVL) RNA binding proteins have been linked to numerous neurological disorders including Alzheimer's disease (AD), yet their function in disease and their targets in the human brain remain unknown. We used high-throughput sequencing combined with UV crosslinking and immunoprecipitation (HITS-CLIP) to globally identify nELAVL binding sites in healthy human brain. We found that nELAVL binds U-rich sequences within 3' untranslated regions (UTRs) and introns, with an enrichment in transcripts important for cell signaling, synaptic transmission and functions related to AD. To investigate dynamic changes in nELAVL-mediated regulation during AD, we generated nELAVL binding maps from brain tissue of patients with advanced AD. nELAVL displayed differential binding between healthy and diseased brain, both within 3'UTRs and introns of critical neuronal transcripts, including AD-related genes. Unexpectedly, the most significant change of nELAVL binding in AD patient brain was a significantly increased association with Y RNAs, a class of non-coding RNAs of largely unknown function. Because the abundance of Y RNAs was unchanged between healthy and AD brain, our results suggest that nELAVL:Y RNP complexes were specifically remodeled during AD progression. The composition of Y RNPs is modulated by external cues like stress, and, consistently, we observed an increased association between nELAVL and YRNAs during acute UV stress in neuroblastoma cells. We propose that the increased nELAVL/Y RNA association during chronic and acute neuronal stress may lead to sequestration of nELAVL, and that the redistribution of nELAVL RNA target binding underlies changes in neuronal mRNA stability and splicing in AD.

Disclosures: C. Scheckel: None. **E. Drapeau:** None. **M. Frias:** None. **J.D. Buxbaum:** None. **R.B. Darnell:** None.

Nanosymposium

110. Alzheimer's Disease: Genetics and Biology I

Location: 152A

Time: Sunday, November 16, 2014, 8:00 AM - 11:30 AM

Presentation Number: 110.12

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

Support: NIH Grant NS080675

NIH Grant P30NS048056

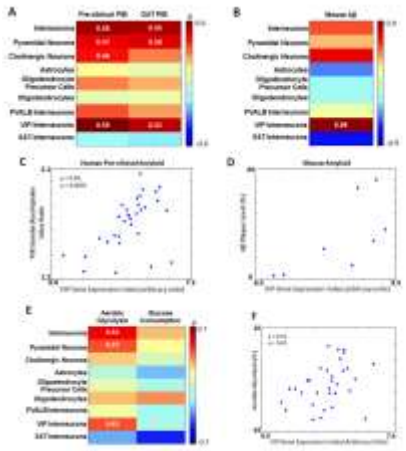
NIH Grant 1P01NS074969

NIH Grant P01NS080675

Title: Amyloid plaques preferentially deposit in VIP interneuron rich brain regions

Authors: *A. MITRA[1], M. S. GOYAL[1], A. Z. SNYDER[1], A. W. BERO[2], T. M. BLAZEY[1], D. M. HOLTZMAN[1], M. E. RAICHLE[1];
[1]Washington Univ. Sch. of Med., Saint Louis, MO; [2]MIT, Cambridge, MA

Abstract: Introduction Amyloid beta (Aβ) accumulates in a stereotypical distribution in the human brain prior to the onset of dementia in Alzheimer's disease (AD). The role that specific neural cell types play in regional vulnerability to Aβ deposition is unknown. We developed a novel method to spatially map cell type-specific gene expression patterns. We then spatially correlated these patterns with amyloid and metabolic imaging in humans and in APP-transgenic mice. **Methods** Metabolic PET glucose and oxygen consumption measurements were acquired in 33 normal young adults (25.4 ± 2.6 yrs). PIB amyloid imaging was obtained in a pre-clinical AD population (N=14; 74.6 ± 7.9 yrs) positive for amyloid deposition, and a second group of 11 individuals (79.7 ± 5.0 yrs) with a clinical diagnosis of AD. Human gene expression data were obtained from the Allen Human Brain Atlas (AHBA). Adult mouse gene expression data were obtained from the BrainStars database. Regional measurements of amyloid plaque burden were obtained in adult Tg2576 AD mice using 3D6B antibody immunoreactivity. **Results** Gene expression patterns specific to GABAergic interneurons spatially correlated with Aβ deposition in both pre-clinical and clinical AD subjects (Fig. 1A). This effect was specifically attributable to VIP interneurons (Fig. 1C). Concordant results were observed in the APP-mouse (Fig. 1B,D). GABAergic interneuron gene expression also highly correlated with aerobic glycolysis (AG), a metabolic marker of neuronal plasticity. Among GABAergic interneurons, VIP interneuron gene expression correlated best with AG (Fig. 1E,F). The correlation between VIP interneuron gene expression and AG was not explained by glucose consumption alone. **Conclusions** Aβ plaques preferentially deposit in VIP interneuron-rich brain regions, both in humans and a mouse model of AD. VIP interneuron gene expression also spatially correlates with AG, suggesting that selective regional vulnerability to Aβ plaque deposition in AD is related to VIP interneuron activity and its relation to cellular mechanisms supporting neuronal plasticity.



Disclosures: **A. Mitra:** None. **M.S. Goyal:** None. **A.Z. Snyder:** None. **A.W. Bero:** None. **T.M. Blazey:** None. **D.M. Holtzman:** None. **M.E. Raichle:** None.

Nanosymposium

110. Alzheimer's Disease: Genetics and Biology I

Location: 152A

Time: Sunday, November 16, 2014, 8:00 AM - 11:30 AM

Presentation Number: 110.13

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

Support: NIA Grant U24 AG21886

NIA Grant U24 AG026395

Title: A rare mutation in UNC5C predisposes to Alzheimer’s disease and increases neuronal cell death

Authors: *M. K. WETZEL;
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Abstract: The overall contribution of rare genetic variants to the risk of late-onset Alzheimer’s disease (LOAD) is unknown. We have identified a rare coding mutation, T835M (rs137875858), in the Netrin receptor UNC5C that segregated with disease in an autosomal dominant pattern in two families enriched for LOAD, and was associated with disease across four case/control cohorts (OR = 2.15, Pmeta= 0.0095). T835M alters a conserved residue in the hinge region of UNC5C, and in vitro studies demonstrate that this mutant leads to increased cell death in several

cell types, including neurons. Furthermore, neurons expressing the T835M UNC5C are more susceptible to Aβ and staurosporine-induced neuronal cell death. Based on these data, combined with the expression pattern of UNC5C in the adult nervous system, we propose one possible mechanism in which T835M UNC5C contributes to risk of Alzheimer's disease by increasing neuronal cell death, particularly in vulnerable regions of the Alzheimer's brain.

Disclosures: **M.K. Wetzel:** A. Employment/Salary (full or part-time):; Genentech.

Nanosymposium

110. Alzheimer's Disease: Genetics and Biology I

Location: 152A

Time: Sunday, November 16, 2014, 8:00 AM - 11:30 AM

Presentation Number: 110.14

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

Support: NIH/NIA 1R01AG042890 to GT

NIH 1UL1RR029876 to NA

Title: Altered membrane lipids, reduced synaptic responses, and increased vulnerability to Aβ oligomers in CNS synapses from transgenic mice with peripheral insulin resistance

Authors: *G. TAGLIALATELA[1], F. TEMPIA[2], Z. WENRU[1], D. TUVDENDORJ[3], H. SALLAM[3], B. TUMURBAATAR[3], F. LAEZZA[2], N. ABATE[3];
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Abstract: Compelling evidence indicates that Type 2 diabetes mellitus (T2DM) increases the risk for Alzheimer's disease (AD). The growing prevalence of these two chronic conditions could be related to the world-wide epidemic of obesity and its metabolic complications, including reduced adipose tissue (AT) production of adiponectin and handing of lipid metabolism (AT dysfunction), increased circulating free fatty acid (FFA), and development of peripheral insulin resistance to glucose disposal (IR). IR is the main metabolic feature preceding and characterizing the pathogenesis of T2DM and has also been observed in patients with AD. It is therefore possible that IR and associated metabolic abnormalities resulting from AT dysfunction explain the heightened risk for AD in patients with or at risk for T2DM. However, the exact mechanisms linking the pathogenesis of IR to CNS alterations found in AD remain elusive. To address this knowledge gap, we have used the At*ENPP1*-Tg transgenic mouse model of AT dysfunction which over-expresses the insulin receptor negative regulator ecto-nucleotide

pyrophosphatase/phosphodiesterase-1 (ENPP1). If challenged with a high-fat diet, At*ENPP1*-Tg recapitulates the metabolic syndrome found in humans with T2DM. We found that brain synaptosomes isolated from high-fat diet fed At*ENPP1*-Tg mice show altered lipid composition, reduced insulin signaling activation, and diminished NMDAR expression and phosphorylation. In the same animals, extracellular field recordings in the CA1 hippocampal region revealed a significant decrease in synaptic responses from CA3 inputs compared to high-fat diet fed wild-type littermates. As a further indication of synaptic deficiency, we found that amyloid beta (Abeta) oligomers bound more readily to brain tissue derived from high-fat diet fed At*ENPP1*-Tg mice compared to wild-type, a phenotype that was prevented by tissue treatment with adiponectin. Given that binding of Abeta oligomers to synapses is a major neurodysfunctional event leading to onset and progression of cognitive decline in AD, our results support the hypothesis that increased synaptic vulnerability to Abeta oligomers and consequent increased risk of AD may be driven by AT dysfunction, a major determinant of IR and risk for T2DM. This new knowledge provides a molecular mechanism for increased risk of AD in people with peripheral IR or T2DM and lays the foundation for the development of future effective therapeutic approaches to reduce the incidence of AD in the at risk population.

Disclosures: G. Taglialatela: None. **F. Tempia:** None. **Z. Wenru:** None. **D. Tuvdendorj:** None. **H. Sallam:** None. **B. Tumurbaatar:** None. **F. Laezza:** None. **N. Abate:** None.

Nanosymposium

111. Motor Neuron Disease Mechanisms and Models

Location: 143A

Time: Sunday, November 16, 2014, 8:00 AM - 11:15 AM

Presentation Number: 111.01

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: A 3-D co-culture system of peripheral motor neurons and Schwann cells demonstrates the myelination process *in vitro*

Authors: *S. HYUNG;
Seongbuk-gu, The Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of

Abstract: Abstract: Unlike the central nervous system, the mammalian peripheral nervous system(PNS) tends to readily regenerate after injury and regain its functions. However, the regeneration capacity of PNS is known to be limited only for injuries of less than 3 cm in length. In this study, we have developed an *in vitro* co-culture model of peripheral motor neurons(PMN) and Schwann cells(SC) to study the mechanisms of axonal regrowth and myelination of

peripheral motor neurons. Peripheral motor neurons were harvested from spinal cord of E14 embryos, and were purified from their neighboring cells using a selective immuno-panning dish. Schwann cells were isolated from the sciatic nerves of 4-day-old CD-1 mice, treated with trypsin solution containing 10mg/ml collagenase A. They were then cultured on matrigel-coated coverslips. The purified PMN were seeded on proliferating Schwann cells which were grown on growth factor reduced matrigel for a 3-D formation. Figure 1 shows the comparison of axonal growth in the PMN mono-culture and PMN-SC co-culture. In presence of SC, the growth cone of PMN not only rapidly generated neuritis but promoted axonal growth. Figure 2 shows that the expression of MAG has highly increased at day 10 and decreased at day 14(data not shown) under strict transcription control. The expression of MBP in the PMN-SC co-culture has been also identified as shown in Figure 2. Myelination controlled by activating transcription factor such as sox10(SRY-related HMGbox-10), as described by Pereira et al.(2012), was confirmed at day 10, and was highly promoted to form myelin sheath at day 14. Figure 3 shows the typical formation of mature sheaths in the PMN-SC co-culture in which the expression of MAG and MBP had topolocally distinctive characteristics. At day 21, MBP was aligned with motor axon expressing BetaIII Tubulin(TujI), while MAG was only found in the region of uncompacted myelin such as the paranodal loops and Schmidt-Lanterman incisures. In summary, it was found that the growth and survival of motor neuron was greatly enhanced by the co-culture with Schwann cell, as compared with motor neuron alone. The myelin formation of PMN was confirmed by the expression of myelin basic protein(MBP) and myelin associated glycoprotein(MAG), the proteins associated with the compact myelin sheath.

Disclosures: S. Hyung: None.

Nanosymposium

111. Motor Neuron Disease Mechanisms and Models

Location: 143A

Time: Sunday, November 16, 2014, 8:00 AM - 11:15 AM

Presentation Number: 111.02

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Muscular Dystrophy Association

Alzheimer's Association

Houston methodist Research Institute

Title: Imbalance in genomic damage and their repair as a common basis for neurodegenerative diseases

Authors: *M. L. HEGDE;
Houston Methodist, Houston, TX

Abstract: Pavana M. Hegde, Erika N. Guerrero, Vishnupriya Borra, KS Rao, Ping Wu, S Mitra and **Muralidhar L. Hegde** (mlhegde@houstonmethodist.org) Departments of Radiation Oncology and Neurology, Houston Methodist Research Institute, affiliate of Weill Medical College of Cornell University, Houston, Texas 77030, USA. Accumulation of genome damage including oxidized bases, single- and double-strand breaks, in affected brain cells has been linked to neurodegenerative diseases whose underlying cause(s) are not completely understood. We recently demonstrated that transition metals iron and copper that accumulate in neurodegenerative brain act as a double-edged sword by both increasing oxidative genome damage and preventing their repair and thus could play a role in accumulation of unrepaired genome damage in neurons leading to cell death (Hegde et al, J Biol Chem 2010: 285, 28812-25). Here, we provide evidence for the first time that RNA binding proteins TDP-43 and FUS/TLS, whose nuclear clearance and simultaneous cytoplasmic deposition is a hallmark feature in Amyotrophic Lateral Sclerosis (ALS) and other neurodegenerative diseases, are required for efficient DNA single and double strand break repair (DSBR), respectively, in neurons. We demonstrate that: (1) TDP-43 stably interacts with DSBR proteins in neuroblastoma SH-SY5Y cells, which was enhanced after treatment with DSB-inducing radiation/bleomycin. (2) TDP-43 is recruited to the DSB sites neuronal cells, and (3) TDP-43's overall as well as nucleus-specific depletion markedly increased accumulation of DSBs in SH-SY5Y cells and sensitized the cells to radiation. It was observed that unlike TDP-43, FUS associates with XRCC1/LigIII complex, which increases after oxidative stress and is involved in base excision and single strand break repair. These results are consistent with the dramatic accumulation of unrepaired DSBs in postmortem brains of ALS-affected human patients and a distinct nuclear clearance of TDP-43/FUS in these affected neurons. Thus deficiency in DNA strand break repair may be a key etiologic factor in neurodegenerative diseases. These results suggest that inhibition of genome damage repair in neuronal genome represents a common basis for neurodegenerative diseases. (Supported by Alzheimer's Association, Muscular Dystrophy Association and Houston Methodist Research Institute)

Disclosures: M.L. Hegde: None.

Nanosymposium

111. Motor Neuron Disease Mechanisms and Models

Location: 143A

Time: Sunday, November 16, 2014, 8:00 AM - 11:15 AM

Presentation Number: 111.03

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Wellcome Trust

MRC UK

ARUK

MNDA

EU Framework 7

Title: ER-mitochondria associations are regulated by the VAPB-PTPIP51 interaction and are disrupted by ALS/FTD-associated TDP-43

Authors: *C. C. MILLER[1], R. STOICA[1], K. DE VOS[3], S. PAILLUSSON[2], K.-F. LAU[4], R. SANCHO[5];
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Abstract: Mitochondria and the endoplasmic reticulum (ER) form tight structural associations and these facilitate a number of essential cellular functions. These include energy metabolism, phospholipid synthesis, mitochondrial biogenesis and trafficking, apoptosis, ER stress responses and autophagy. However, the mechanisms by which regions of the ER become tethered to mitochondria are not properly known. Understanding these mechanisms is not just important for comprehending fundamental physiological processes but also for understanding pathogenic processes in some disease states. In particular, disruption to ER-mitochondria associations is linked to some neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and fronto-temporal dementia and associated amyotrophic lateral sclerosis (FTD/ALS). Here we show that the ER-resident protein VAPB interacts with the outer mitochondrial membrane protein PTPIP51 to regulate ER-mitochondria associations. Moreover, we demonstrate that TDP-43, a protein pathologically linked to FTD and ALS perturbs ER-mitochondria interactions and that this is associated with a disruption to the VAPB-PTPIP51 interaction and cellular Ca2+ homeostasis. Finally, we show that overexpression of TDP-43 leads to activation of glycogen synthase kinase-3β (GSK-3β) and that GSK-3β regulates the VAPB-PTPIP51 interaction. Our results describe a new pathogenic mechanism for TDP-43 in FTD/ALS.

Disclosures: C.C. Miller: None. **R. Stoica:** None. **K. De Vos:** None. **S. Paillusson:** None. **K. Lau:** None. **R. Sancho:** None.

Nanosymposium

111. Motor Neuron Disease Mechanisms and Models

Location: 143A

Time: Sunday, November 16, 2014, 8:00 AM - 11:15 AM

Presentation Number: 111.04

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: W81XWH-09-1-0245

Title: Astrocyte mediated toxicity leads to motor neuron death in Spinal Muscular Atrophy

Authors: S. PHANI[1], A. JACQUIER[2], D. PAPADIMITRIOU[2], V. LEVERCHE[2], R. PRADHAN[2], S. KARIYA[2], U. MONANI[2], *D. B. RE[2], S. PRZEDBORSKI[2];
[1]Pathology, [2]Neurol., Columbia Univ., NEW YORK, NY

Abstract: Spinal Muscular Atrophy (SMA) is the leading genetic cause of death in infants and toddlers. In children with SMA, loss of motor neurons in the spinal cord triggers rapidly progressive paralysis, and death in the most severe cases. SMA is caused by a mutation in the Survival of Motor Neuron 1 (SMN1) gene, resulting in diminished production of SMN protein in all cells and tissue. However, the mechanism by which this mutation and reduction in protein causes motor neuron death is unclear. Our previous studies from Amyotrophic Lateral Sclerosis (ALS) have shown the importance of astrocyte mediated toxicity in motor neuron disease, raising the hypothesis that non-neuronal cells may play an important role in SMA related motor neuron degeneration. In order to test this hypothesis, embryonic spinal cord motor neurons were cultured from SMA Type 1 mice in the presence or absence of astrocytes. In these studies, we show that Type 1 MNs die in the presence of Type 1 or WT astrocytes, however, they do not die in the absence of astrocytes. These findings suggest a combination of both cell autonomous and non-cell autonomous components in the MN pathology of SMA. Moving forward, we focused on the combination of SMA MN/SMA Astro, the condition we thought to be most physiologically relevant. In order to determine the mechanism of cell death, we used biochemical inhibitors of individual death pathways, as well as immunocytochemical analysis of cell death markers including TUNEL and Ethidium Homodimer (EtHD). Our initial findings suggest that the blockade of Caspase 8 activity attenuates the MN death seen in our in vitro Type 1 model of SMA. Interestingly, through western blot analysis, we also find a marked increase of caspase 8 in SMA astrocytes when compared to WT astrocytes. Additionally, our results suggest a pronounced increase in TUNEL and EtHD labeling in SMA motor neurons compared to wild type controls. These data suggest an apoptosis-like mechanism that may be responsible for the motor neuron death observed in SMA. Non-Cell Autonomous cell death is a prevalent mechanism in neurodegenerative disorders. Here we show the role that astrocytes play to

contribute to motor neuron death in SMA. We suggest potential signaling pathways that may be triggered by non-motor neurons that lead to the eventual pathology seen in SMA.

Disclosures: **S. Phani:** None. **D.B. Re:** None. **A. Jacquier:** None. **D. Papadimitriou:** None. **V. LeVerche:** None. **R. Pradhan:** None. **S. Kariya:** None. **U. Monani:** None. **S. Przedborski:** None.

Nanosymposium

111. Motor Neuron Disease Mechanisms and Models

Location: 143A

Time: Sunday, November 16, 2014, 8:00 AM - 11:15 AM

Presentation Number: 111.05

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: The role of nuclear transport defects in the pathogenesis of amyotrophic lateral sclerosis

Authors: ***K. ZHANG**[1], C. DONNELLY[1], A. HAEUSLER[2], J. WANG[2], R. SATTLER[1], J. ROTHSTEIN[1], T. LLOYD[1];
[1]Dept. of Neurol. and Brain Sci. Inst., Baltimore, MD; [2]Dept. of Biochem. and Mol. Biol. Johns Hopkins University, Bloomberg Sch. of Publ. Hlth., Baltimore, MD

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive, uniformly fatal neurodegenerative disease of motor neurons that is accompanied by frontotemporal dementia (FTD) in approximately 15% of patients. Expansion of GGGGCC (G4C2) hexanucleotide repeats in the first intron of the C9ORF72 gene is the most common genetic cause of familial ALS and FTD. Increasing evidence suggests that the G4C2 hexanucleotide repeat expansion (HRE) acts in a toxic gain-of-function mechanism. Using a Drosophila model of ALS that expresses 30 G4C2 repeats (Xu et al, PNAS, 2013), we performed a candidate-based screen to identify genetic modifiers of HRE-mediated neurodegeneration. This screen of ~400 candidate genes identified several strong modifiers, of which the most potent suppression was mediated by a gain-of-function allele of the RanGAP gene. RanGAP encodes Ran GTPase activating protein, a critical regulator of nucleocytoplasmic transport. Interestingly, genetic manipulations that enhance nuclear import or inhibit nuclear export suppress neurodegeneration. Furthermore, overexpression of G4C2 repeats disrupt nuclear import of GFP containing a nuclear localization signal (NLS), and this defect can be mitigated by Antisense Oligonucleotides or small molecules that bind to the HRE. Consistent with our hypothesis of nuclear import disruption in ALS, the nuclear protein TAR DNA-binding protein 43 (TDP-43) accumulates in the cytoplasm and is lost from the nucleus in the majority of ALS cases, including ALS caused by the C9ORF72 HRE.

Indeed, the fly homologue of TDP-43 called TBPH accumulates in cytoplasm of G4C2-expressing photoreceptors with aging. Interestingly, iPS neurons derived from C9-ALS patients exhibit perturbed Ran protein gradients, an indication of defective nuclear import. We hypothesize that generalized nucleocytoplasmic transport defects might underlie the cytoplasmic accumulation of TDP-43 and contribute to the pathogenesis of ALS/FTD.

Disclosures: **K. Zhang:** None. **C. Donnelly:** None. **A. Haeusler:** None. **J. Wang:** None. **R. Sattler:** None. **J. Rothstein:** None. **T. Lloyd:** None.

Nanosymposium

111. Motor Neuron Disease Mechanisms and Models

Location: 143A

Time: Sunday, November 16, 2014, 8:00 AM - 11:15 AM

Presentation Number: 111.06

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Department of Veterans Affairs [Merit Review Grant #1147891 to B.K. and CDA2 Award #I01BX007080 to N.L.]

and National Institutes of Health [R01NS064131 to B.K.

Title: The tau tubulin kinases TTBK1/2 promote accumulation of pathological TDP-43

Authors: ***B. C. KRAEMER**[1], N. LIACHKO[2], P. MCMILLAN[2], T. STROVAS[2]; [1]GRECC, Veterans Affairs Puget Sound Hlth. Care Syst., Seattle, WA; [2]VA PSHCS, Seattle, WA

Abstract: Pathological aggregates of phosphorylated TDP-43 characterize amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD-TDP), two devastating groups of neurodegenerative disease. Kinase hyperactivity may be a consistent feature of ALS and FTLD-TDP, as phosphorylated TDP-43 is not observed in the absence of neurodegeneration. By examining changes in TDP-43 phosphorylation state, we have identified kinases controlling TDP-43 phosphorylation in a C. elegans model of ALS. In this kinome-wide survey, we identified homologs of the tau tubulin kinases 1 and 2 (TTBK1 and TTBK2), which were also identified in a prior screen for kinase modifiers of TDP-43 behavioral phenotypes. Using refined methodology, we demonstrate TTBK1 and TTBK2 directly phosphorylate TDP-43 in vitro and promote TDP-43 phosphorylation in mammalian cultured cells. TTBK1/2 overexpression drives phosphorylation and relocalization of TDP-43 from the nucleus to large cytoplasmic inclusions

reminiscent of neuropathologic changes in disease states. Furthermore, protein levels of TTBK1 and TTBK2 are increased in frontal cortex of FTLD-TDP patients, and TTBK1 and TTBK2 co-localize with TDP-43 inclusions in ALS spinal cord. These kinases may represent attractive targets for therapeutic intervention for TDP-43 proteinopathies such as ALS and FTLD-TDP.

Disclosures: B.C. Kraemer: None. **P. McMillan:** None. **N. Liachko:** None. **T. Strovas:** None.

Nanosymposium

111. Motor Neuron Disease Mechanisms and Models

Location: 143A

Time: Sunday, November 16, 2014, 8:00 AM - 11:15 AM

Presentation Number: 111.07

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Authors acknowledge support by grants from UAMS startup funds and the College of Medicine Research Council. Also, this research is funded by a pilot study award from the Center for Translational Neuroscience, NIGMS IDeA Program award P20 GM103425-10.

Title: Development of mouse model for a newly discovered mutant profilin1 in fALS patients

Authors: *M. KIAEI, S. YADAV;
Neurobiology/Dev Sci., Univ. of Arkansas for Med. Sci., Little Rock, AR

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that mainly affects motor neurons in cortex, brainstem and spinal cord. The mechanism of neuronal degeneration and muscle atrophy in ALS is poorly understood. Existing models have little utility in studies to understand the mechanism of new mutant molecules like profilin1 that was found to be linked to fALS. Recently, five mutations in profilin1 (*PFN1*) gene (ALS18) were identified to be linked to a subpopulation of fALS patients which had none of the previously known mutated genes in fALS (Wu et al. 2012). Whether profilin1 mutations in this group of ALS patients is a cause of ALS, remain unknown. Identification of *PFN1* mutation in human ALS patients with approximately 10 years earlier on average age of onset than other ALS patients and common clinical limb onset makes a strong case for its involvement but doesn't automatically confer that it is the cause. Profilin1 has not been linked to any disease or syndrome in the past with an exception that the deletion of a locus in chromosome 17p13.3 (*PFN1* is located on this locus) is associated with Miller-Dieker syndrome, which is an abnormal brain development known as type I Lissencephaly (Miller 1963). This is very interesting and not fully understood. To address the

cause and effect, and mechanism of profilin1 neurotoxicity for ALS, we developed transgenic mice that overexpress human profilin1 mutation and examined the animals for ALS-like phenotype and for the mechanism(s) of mutant PFN1 neurotoxicity. We will present the evidence from our study that we have successfully developed transgenic mice overexpressing mutant human PFN1. Our profilin1 transgenic mice are viable and appear normal at birth and healthy enough to breed and generate viable offspring. Mutant PFN1 mice develop ALS-like phenotypes such as hindlimb fine tremor and clasping, gait abnormality leading to low body profile, reduced stride length, gradual weakness and atrophy in muscle of limbs, kyphosis, significant weight loss toward later part of the disease, and significantly reduced life-span. In summary, we have developed a new mouse model overexpressing a novel mutation in human profilin1 gene found in fALS . Overexpression of mutant human PFN1 in our mice resulted in the development of ALS-like phenotypes. To our knowledge this model is the first to be produced and develop symptoms and signs that resembles ALS. This model potentially has utility in investigation of mutant profilin1 neurotoxicity in motor neurons and how it causes ALS. This model is expected to be useful for testing therapeutic strategies for development of therapy for ALS.

Disclosures: **M. Kiaei:** None. **S. Yadav:** None.

Nanosymposium

111. Motor Neuron Disease Mechanisms and Models

Location: 143A

Time: Sunday, November 16, 2014, 8:00 AM - 11:15 AM

Presentation Number: 111.08

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01 NS060698

Title: Optineurin is an autophagy receptor for damaged mitochondria in parkin-mediated mitophagy that is disrupted by an ALS-linked mutation

Authors: ***Y. C. WONG**, E. HOLZBAUR;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Mitophagy is a cellular quality control pathway in which the E3 ubiquitin ligase parkin targets damaged mitochondria for degradation by autophagosomes. We examined the role of optineurin in mitophagy, as mutations in optineurin are causative for Amyotrophic lateral sclerosis (ALS) and glaucoma, diseases in which mitochondrial dysfunction has been implicated. We used live cell imaging to demonstrate the parkin-dependent recruitment of optineurin to

mitochondria damaged by depolarization or reactive oxygen species (ROS) production. Parkin induced stabilized binding of optineurin to damaged mitochondria via its ubiquitin binding domain (UBAN); in the absence of parkin, optineurin transiently localizes to damaged mitochondrial tips. Double FYVE-containing protein 1 (DFCP1) puncta are selectively recruited to optineurin-labeled mitochondria to initialize autophagosome formation, followed by autophagic engulfment of optineurin-labeled mitochondria. Autophagosome recruitment is accelerated by increased optineurin expression, while either depletion of endogenous optineurin or expression of an ALS-associated mutation in the UBAN domain inhibit mitochondrial engulfment. Our study is the first to demonstrate a role for optineurin as an autophagy receptor in parkin-mediated mitophagy, and implicates defects in a single pathway leading to neurodegenerative diseases with distinct pathologies.

Disclosures: **Y.C. Wong:** None. **E. Holzbaur:** None.

Nanosymposium

111. Motor Neuron Disease Mechanisms and Models

Location: 143A

Time: Sunday, November 16, 2014, 8:00 AM - 11:15 AM

Presentation Number: 111.09

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Fondazione Telethon, Italy GGP07073

Fondazione AriSLA, Italy

Fondation AFM-Telethon, France

Regione Lombardia, Italy

Università degli Studi di Milano, Italy

Ministero della Salute, Italy

Title: Inhibition of dynein-mediated retrograde transport reduces aggregation of misfolded protein responsible for motoneuron diseases

Authors: *A. POLETTI, R. CRISTOFANI, P. RUSMINI, V. CRIPPA, E. GIORGETTI, A. BONCORAGLIO, M. E. CICARDI;
Dept. di Scienze Farmacologiche e Biomolecolari, Univ. Degli Studi Di Milano, Milano, Italy

Abstract: Motor neuron diseases, like spinobulbar muscular atrophy (SBMA) and amyotrophic lateral sclerosis (ALS) are characterized by the presence of inclusions o aggregates of proteinaceous materials. In SBMA, aggregates contain mutant androgen receptors (AR) with an elongated polyglutamine tract (ARpolyQ), responsible for disease, while in ALS aggregates contain TDP43, ubiquilin, optineurin, etc. Exceptions are familial ALS forms linked to superoxide dismutase 1 (SOD1) mutations, in which aggregates are composed of mutant SOD1. In general, protein aggregation is due to generation of aberrant protein conformations (misfolding). Thus, in neuronal cells, the protein quality control (PQC) system may be insufficient to correctly remove the misfolded proteins. The PQC system requires the activities of efficient chaperones and of the degradative systems ubiquitin-proteasome (UPS) and autophagy. After misfolded protein recognition by chaperones, the dynein motor complex plays a crucial role to efficiently remove these species via autophagy transporting them to autophagosome and assisting autophagosome-lysosome fusion. We found that in immortalized motoneuronal NSC34 cells treated with trehalose to induce autophagy, a selective inhibitor of dynein (EHNA) drastically reduced activated autophagy. In addition, in NSC34 cells expressing ARpolyQ dynein was sequestered into ARpolyQ aggregates. When we perturbed dynein function with EHNA in NSC34 cells expressing ARpolyQ, mutant SOD1 or a truncated TDP43 form, we unexpectedly found a great reduction of mutant protein aggregates, even in presence of an autophagy inhibitor (3-MA), but not of a proteasome inhibitor (MG132). In fractionation studies we found that EHNA increased the ARpolyQ levels in PBS and Triton-X100 fractions. Surprisingly, we found that ENHA effects were paralleled by an increased expression of BAG1, a co-chaperone which routes misfolded proteins to UPS, but not of BAG3, required for autophagy. The decreased BAG3/BAG1 ratio is suggestive of a prevalence of UPS functions when dynein activity is impaired. Thus, dynein blockage results in autophagy alteration, but also to a reduced aggregation of misfolded proteins,, a phenomenon that may occurs via an increase in their solubility and the induction of UPS functions.

Disclosures: **A. Poletti:** None. **R. Cristofani:** None. **P. Rusmini:** None. **V. Crippa:** None. **E. Giorgetti:** None. **A. Boncoraglio:** None. **M.E. Cicardi:** None.

Nanosymposium

111. Motor Neuron Disease Mechanisms and Models

Location: 143A

Time: Sunday, November 16, 2014, 8:00 AM - 11:15 AM

Presentation Number: 111.10

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NS066888-01

Title: Defining spinal muscular atrophy gene networks in *Drosophila*

Authors: *E. M. MCNEILL[1], T. YOKOKURA[2], J. YELICK[1], T. CHOBANYAN[1,2], D. VAN VACTOR[1,2];
[1]Cell Biol., Harvard Med. Sch., Boston, MA; [2]Okinawa Inst. of Sci. and Technol., Okinawa, Japan

Abstract: Spinal Muscular Atrophy (SMA) refers to a group of recessive genetic diseases characterized by motorneuron degeneration and muscle weakness. SMA has been tied to mutations in the survival motor neuron (SMN) gene, but the molecular mechanisms underlying the specific impact of disease on motor neurons and neuromuscular connections remain largely uncharacterized. Here we demonstrate that mutations in the highly conserved *Drosophila* ortholog of human SMN1 result in ultrastructural defects in the formation of the synapse at the neuromuscular junction (NMJ). We examine the role of the canonical signaling pathways BMP and FGF in the synaptic phenotype of SMN loss. Additionally, we identify a novel peripheral axon degeneration phenotype in the *Drosophila* disease model. We examine the tissue-specific requirement for SMN in the axonal phenotype as well as the genetic mechanisms underlying axonal degeneration. The results of this work identify novel phenotypes similar to those observed in human disease in the *Drosophila* model of SMN. This genetic model thus enables us to examine the molecular mechanisms underlying the disease, more closely defining the genetic pathways involved in these disease phenotypes and identifying potential therapeutic targets for SMA.

Disclosures: E.M. McNeill: None. **T. Yokokura:** None. **J. Yelick:** None. **T. Chobanyan:** None. **D. Van Vactor:** None.

Nanosymposium

111. Motor Neuron Disease Mechanisms and Models

Location: 143A

Time: Sunday, November 16, 2014, 8:00 AM - 11:15 AM

Presentation Number: 111.11

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Differential roles of RNA-binding, self-assembly and familial ALS mutations of FUS in the retinal degeneration of transgenic *Drosophila* melanogaster overexpressing human FUS

Authors: *T. HASHIMOTO[1], T. MATSUMOTO[2], H. KUNUGI[2], K. MATSUKAWA[1], H. UCHIGAMI[1], T. CHIHARA[3], M. MIURA[3], T. WAKABAYASHI[1], T. IWATSUBO[1]; [1]Dept. of Neuropathology, [2]Dept. of Neuropathology and Neurosci., [3]Dept. of Genet., The Univ. of Tokyo, Tokyo, Japan

Abstract: Fused in sarcoma (FUS) has been identified as the causative gene for autosomal-dominant familial amyotrophic lateral sclerosis (ALS) type 6. Wild-type FUS is predominantly localized in the nuclei of neurons, whereas cytoplasmic FUS-immunoreactive inclusions are observed in degenerating neurons of sporadic or FUS-linked familial ALS (fALS), or frontotemporal lobar degeneration (FTLD). However, the molecular mechanism whereby FUS leads to neurodegeneration is still unclear. To elucidate the pathogenic mechanisms of FUS, we generated transgenic Drosophila melanogaster (tg fly) overexpressing human FUS in retinal photoreceptor neurons using the GAL4/UAS system under the control of the GMR driver, and found that overexpressed wild-type FUS is predominantly localized in the nuclei. Wild-type FUS tg flies exhibited ommatidial disorganization, vacuolar degeneration and thinning of the retina. Strikingly, these degenerative phenotypes were completely normalized by deletion of the RNA recognition motif (RRM) of FUS that abolishes the RNA binding ability. We next examined the effects of fALS mutations in FUS (R514G, R521C or P525L) and found that overexpressed mutant FUS was localized both in the nucleus and cytoplasm. FALS mutant FUS tg flies exhibited more severe degenerative phenotypes than those in wild-type FUS tg flies. Remarkably, the deletion of RRM did not normalize the retinal degeneration caused by fALS mutant FUS. These results suggested that wild-type FUS causes neurodegeneration chiefly through its RNA-binding ability, whereas the pathogenic effect of fALS mutant FUS involves RNA-binding independent mechanisms. The N-terminal region of FUS harbors the low-complexity/prion-like (LC) domain, which has been reported to be necessary for its self-assembly; indeed, allS mutant FUS, in which 27 tyrosine residues in the LC domain are replaced with serine was shown to be incapable of the self-assembly. We found that overexpression of allS mutant FUS, either on wild-type basis or harboring fALS-linked mutations, did not elicit neurodegenerative phenotype in the retina, suggesting that the integrity of the LC domain of FUS is required for the neurodegenerative mechanisms caused by wild-type or fALS mutant FUS. We confirmed that deletion of the LC domain did not alter the RNA-binding ability of FUS in vitro. Taken together, we propose that the RNA-binding ability, self-assembly supported by the LC domain, and fALS mutation differentially affect the neurodegeneration caused by FUS. We thank Drs. Masato Kato, Leeju C. Wu and Steven L. McKnight of University of Texas Southwestern Medical Center for sharing the plasmid encoding allS derivative of FUS.

Disclosures: T. Hashimoto: None. **T. Matsumoto:** None. **H. Kunugi:** None. **K. Matsukawa:** None. **H. Uchigami:** None. **T. Chihara:** None. **M. Miura:** None. **T. Wakabayashi:** None. **T. Iwatsubo:** None.

Nanosymposium

111. Motor Neuron Disease Mechanisms and Models

Location: 143A

Time: Sunday, November 16, 2014, 8:00 AM - 11:15 AM

Presentation Number: 111.12

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Les Turner ALS Foundation

Wenske Foundation

Title: Characterization of innate and adaptive immune responses in the hSOD1G93A-MCP1-CCR2 triple transgenic ALS mouse

Authors: *P. OZDINLER[1], J. H. JARA[2], C. FARRIS[3], R. MILLER[2], J. TRIMARCHI[3];
[1]Dept. of Neurol., Northwestern University, Feinberg Sch. of Med., CHICAGO, IL;
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Abstract: Amyotrophic lateral sclerosis (ALS) is a complex neurodegenerative disease characterized by progressive degeneration of the motor neuron circuitry. Multiple studies have revealed the involvement of innate and adaptive immune responses in both the spinal cord and motor cortex of ALS patients, and in mouse models of ALS. . However, their role in ALS pathology, and specially their association to motor neuron death are not completely elucidated. Secretion of the cytokine MCP1 (monocyte chemoattractant protein-1) has been detected in both cerebrospinal fluid and spinal cord of ALS patients and mouse models of ALS. Additionally, MCP1-mediated recruitment of CCR2 (CC chemokine receptor 2) + monocytes is supported by decreased levels of CCR2 + monocytes in the blood of ALS patients. The purpose of this study is to understand the cellular components and the molecular basis of innate and adaptive immune response in ALS using a novel hSOD1G93A-MCP1-CCR2 triple transgenic ALS mouse model. In this model, MCP1+ and CCR2+ cells are genetically labeled with mRFP (monomeric red fluorescent protein) and eGFP (enhanced green fluorescent protein), respectively. This allows for visualization and isolation based on their fluorescent character. Our intent is not to characterize the MCP1 and CCR2 system in ALS, but rather to use their expression pattern as a bait to genetically label cells of interest. For this purpose, we evaluate MCP1+ and CCR2+ expression at different stages of disease in different regions of the cerebral cortex and spinal cord where neurodegeneration is observed. Our results reveal high levels of MCP1+ cells that belong to microglia lineage in the motor cortex at pre-symptomatic stage, and Interestingly, CCR2+ cells express markers of infiltrating monocytes. Furthermore, fluorescence-activated cell sorting (FACS) has allowed us to isolate MCP1+ and CCR2+ cells from the complex and heterogeneous

structure of the brain and spinal cord for microarray analysis. Evaluating the cellular identity together with their transcription profile has the potential to reveal details of the molecular controls over initiation and progression of immunity in ALS. Identification of cellular mechanisms involved in the immunologic response to vulnerable motor neurons will help reveal novel therapeutic targets for building effective treatment strategies.

Disclosures: P. Ozdinler: None. **J.H. Jara:** None. **C. Farris:** None. **R. Miller:** None. **J. Trimarchi:** None.

Nanosymposium

111. Motor Neuron Disease Mechanisms and Models

Location: 143A

Time: Sunday, November 16, 2014, 8:00 AM - 11:15 AM

Presentation Number: 111.13

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS065895

Title: Gnx4728, a small molecule drug modulator of mitochondrial permeability transition, is therapeutic in a mouse model of amyotrophic lateral sclerosis

Authors: *L. J. MARTIN[1], M. WONG[1], F. DRAGHI[2], S. PLYTE[2], Q. CHANG[1];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurological disorder in humans characterized by progressive degeneration of skeletal muscles and motor neurons in spinal cord, brainstem, and cerebral cortex resulting in paralysis and respiratory insufficiency. ALS can be inherited, but most cases are not associated with a family history of the disease. Mitochondria have been implicated in the pathogenesis, but definitive genetic proof of their role in causal mechanisms is lacking. There are no cures or effective treatments for ALS, and mitochondrial-based concepts of disease mechanisms have not yet offered new therapeutic approaches for ALS. Identification of new clinically translatable disease mechanism-based molecular targets and small molecule drug candidates are needed for ALS patients. We tested the hypothesis in an animal model that drug modulation of the mitochondrial permeability transition pore (mPTP) is therapeutic in ALS. A prospective randomized placebo-controlled drug trial was done in a transgenic mouse model of ALS. We explored GNX4728 as a therapeutic drug. GNX4728 inhibits mPTP opening and increases mitochondrial calcium retention capacity. Chronic systemic

treatment of G37R-human mutant superoxide dismutase-1 (hSOD1) transgenic mice with GNX4728 resulted in major therapeutic benefits. GNX4728 slowed disease progression and significantly improved motor function in G37R-hSOD1 mice. The survival of G37R-hSOD1 mice was improved significantly by GNX4728 treatment as evidenced by a nearly 2-fold extension of lifespan. GNX4728 protected against motor neuron degeneration and mitochondrial degeneration and preserved neuromuscular junction innervation in G37R-hSOD1 mice. This work demonstrates that a mPTP-acting drug has major disease-modifying efficacy in a preclinical mouse model of ALS and establishes the mPTP and mitochondrial calcium retention as targets for ALS drug development and therapeutics.

Disclosures: L.J. Martin: None. **M. Wong:** None. **F. Draghi:** None. **S. Plyte:** None. **Q. Chang:** None.

Nanosymposium

112. Autism: Physiology and Systems III

Location: 146C

Time: Sunday, November 16, 2014, 8:00 AM - 10:15 AM

Presentation Number: 112.01

Topic: C.06. Developmental Disorders

Support: NIH/NINDS R01 NS048527-08

NIH/NCATS grant UL1 TR 000424-06

NIH/NCATS grant P41 EB015909-13

Autism Speaks Foundation

Title: Cerebellar grey matter correlates with early language delay in autism

Authors: *A. M. D'MELLO[1], D. M. MOORE[1], D. CROCETTI[2], S. H. MOSTOFSKY[2], C. J. STOODLEY[1];
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Abstract: Background: Early language delay is one of the earliest indicators of autism spectrum disorder (ASD) and is correlated with later cognitive and behavioral outcomes. Previous structural neuroimaging findings showed that language impaired children with ASD had a smaller cerebellar vermis and abnormal lateralization in the cerebellum compared to non-language impaired children with ASD. Both functional and structural neuroimaging suggest that

the cerebellum, particularly lobules VI, VII (Crus I & II) and VIII, is involved in speech processing and production, language learning, phonological storage, and verbal working memory. This provides an important framework for interpreting cerebellar findings within ASD. Objective: We investigated cerebellar regional grey matter (GM) differences in ASD children with early language delay (ELD) and without ELD and compared these groups to typically developing (TD) children. Our goal was to determine whether patterns of cerebellar GM could differentiate children with a history of ELD from those without ELD. Methods: Voxel-based Morphometry (VBM) was used to examine GM differences in ASD children with ELD (mean age = 10.23±1.18 years) and ASD children without ELD (mean age = 11.05±1.64 years) compared with age-matched TD children (mean age = 10.4±1.5 years). Within the ASD groups, Autism Diagnostic Observation Schedule (ADOS) and Autism Diagnostic Interview (ADI) scores were entered into a full factorial model to determine cerebellar regions in which the relationship between GM and autism diagnostic scores differed by group (group x score interactions). Results: VBM revealed reduced GM in ELD children in the bilateral anterior cerebellum and bilateral Crus I/Crus II compared to TD children. VBM revealed increased GM in non-ELD children in right lobules I-IV and right lobule IX compared to TD children. Within ASD, ELD children had reduced GM in left lobule VII (Crus I), vermis VI, and right lobules IV/V when compared to non-ELD children. In addition, there was a significant group x score interaction for ADOS Social Interaction, Communication, and Repetitive behavior scores that converged on left Crus I/II. Conclusions: The cerebellum was the only region in the brain which differentiated ASD children with ELD from ASD children without ELD in left Crus I, vermis VI, and right lobules I-IV. In addition, group x score interactions converged on left Crus I/II, suggesting that GM patterns in this region structurally and behaviorally differentiate ASD children with and without early language delay.

Disclosures: **A.M. D'Mello:** None. **D.M. Moore:** None. **D. Crocetti:** None. **S.H. Mostofsky:** None. **C.J. Stoodley:** None.

Nanosymposium

112. Autism: Physiology and Systems III

Location: 146C

Time: Sunday, November 16, 2014, 8:00 AM - 10:15 AM

Presentation Number: 112.02

Topic: C.06. Developmental Disorders

Support: Japan Grants-in-Aid for Scientific Research 25242077 and 23111005 to T. T

Japan Grants-in-Aid for Scientific Research 25123716 and 25117006 to K.H.

Japan Grants-in-Aid for Scientific Research 2420007 to M.W.; 25000015 to M.K.

CREST Japanese Science and Technology Agency (T. T.)

MEXT, Japan (M.K.)

the Global COE Program (M.K.)

the Nancy Lurie Marks Family Foundation (S.W.)

Title: Deficits in cerebellar plasticity and motor learning in a copy number variation mouse model of autism spectrum disorder

Authors: *C. PIOCHON[1], D. H. SIMMONS[1], A. D. KLOTH[2], G. GRASSELLI[1], H. K. TITLEY[1], H. NAKAYAMA[3], K. HASHIMOTO[4], V. WAN[1], T. EISSA[1], J. NAKATANI[5], A. CHERSKOV[2], T. MIYAZAKI[6], M. WATANABE[6], T. TAKUMI[7], M. KANO[8], S. S. H. WANG[2], C. HANSEL[1];
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Abstract: Autism Spectrum Disorder (ASD) is defined by two characteristic symptoms, deficits in social interaction and occurrence of repetitive behaviors. An often overlooked, additional feature common to autism is impairment of motor control and motor learning. These observations suggest that dysfunction of the cerebellum might contribute to motor_and possibly non-motor_ASD symptoms. To assess cerebellar abnormalities in ASD, we studied motor behaviors and cerebellar synaptic plasticity in a mouse model for the human 15q11-13 duplication, which is one of the most frequent and also one of the most penetrant genetic abnormalities in autism and which is associated with motor problems in patients. In mice with a paternally inherited duplication (patDp/+), ASD-resembling behaviors, including poor social interaction and behavioral inflexibility, have been described. Here, we found that delay eyeblink conditioning_a form of cerebellum-dependent associative motor learning_as well as gait control are impaired in these mice. We next examined putative synaptic learning correlates in cerebellar slices. In patDp/+ mice, long-term potentiation (LTP) at parallel fiber_Purkinje cell synapses was intact, but long-term-depression (LTD) was prevented at these synapses and LTP was induced instead. Similarly, the elimination of surplus climbing fiber (CF) inputs, a synaptic pruning process that provides a critical step in the development of cerebellar circuits, is impaired. Thus, the 15q11-13 duplication affects synaptic plasticity in the developing and adult cerebellum as

well as cerebellum-dependent motor learning. These findings identify cerebellar abnormalities that may contribute to motor problems and possibly non-motor symptoms in autism, and point to deficits in synaptic pruning and plasticity as potential causes for abnormal synapse and circuit development throughout the autistic brain.

Disclosures: **C. Piochon:** None. **D.H. Simmons:** None. **A.D. Kloth:** None. **G. Grasselli:** None. **H.K. Titley:** None. **H. Nakayama:** None. **K. Hashimoto:** None. **V. Wan:** None. **T. Eissa:** None. **J. Nakatani:** None. **A. Cherskov:** None. **T. Miyazaki:** None. **M. Watanabe:** None. **T. Takumi:** None. **M. Kano:** None. **S.S.H. Wang:** None. **C. Hansel:** None.

Nanosymposium

112. Autism: Physiology and Systems III

Location: 146C

Time: Sunday, November 16, 2014, 8:00 AM - 10:15 AM

Presentation Number: 112.03

Topic: C.06. Developmental Disorders

Title: Cerebellar involvement in autism spectrum disorders and cognitive ability

Authors: ***M. M. PARK**[1], J. P. LERCH[2], A. N. VOINESKOS[1], M. CHAKRAVARTY[1]; [1]Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada; [2]Program in Neurosci. and Mental Hlth., Hosp. for Sick Children, Toronto, ON, Canada

Abstract: Introduction Cerebellar contributions to autism spectrum disorders (ASD) have been heterogeneous across neuroimaging studies, despite converging evidence from genetic, molecular, and post-mortem studies. Our goal was to identify patterns of cerebellar changes in the Autism Brain Imaging Data Exchange (ABIDE) cohort using a novel method for automated segmentation of the cerebellum. We performed a large-scale analysis across the cerebellar subregions to identify possible neuroimaging biomarkers for differentiation between controls and ASD subjects, and the impact of cognitive ability on cerebellar volumes. Methods Data was obtained from the ABIDE consortium, consisting of MRI brain scans from 17 sites around the world. Images were processed using a recently developed automatic segmentation algorithm of the cerebellum and its subregions (MAGeT Brain). Subjects were included in the analysis if their automated segmentations passed stringent quality control by an expert observer (Fig A). Volumes of these subregions were analyzed per site in a linear regression to quantify effects of diagnosis and Full Scale IQ (FIQ), accounting for age, sex, and intracranial volume (ICV) as covariates. Results from each site were pooled in a meta-analysis using a random-effects model. Results 709 subjects (376 controls, 333 ASD) were analyzed after quality control. Volumes were

not significantly different between groups (p range: 0.2-1), while we observed significant effects of FIQ on subregion volumes (Fig B,C). FIQ was lower in ASD subjects (p= 0.005, b=-5.04), and interaction of diagnosis and FIQ was significant for left inferior posterior (p=0.005, b=-21.5) and left anterior lobes (p=0.04, b=-7.9). Conclusions Here we attempted to address heterogeneity of cerebellar contributions in ASD. We found no difference between controls and ASD. Observed effects of FIQ are present for both controls and ASD. Our results suggest 1. Abnormal cerebellar morphology may only be implicated in ASD subjects with low FIQ, and 2. Cerebellar subregions differentially influence cognitive ability.

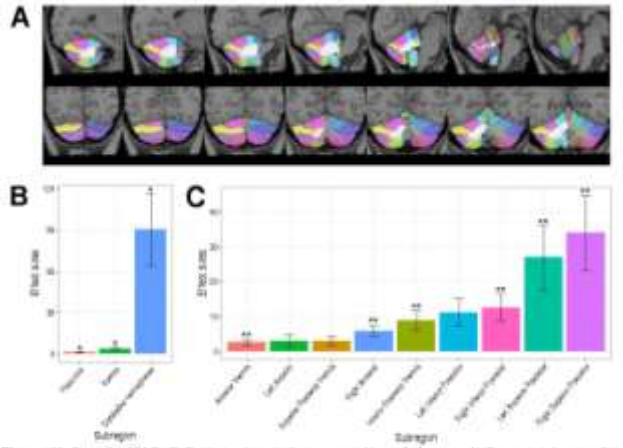

Figure A) Sample MAGeT Brain automated segmentation of the cerebellum passing quality control for sagittal and coronal views. B, C) Meta-analysis: effect sizes (beta) of FIQ on cerebellar subregion volumes, based on two anatomical subdivisions of descending hierarchy: A) Evolutionary divisions, B) Lobar divisions within vermis and cerebellar hemispheres. Asterisks denote significance after Bonferroni correction for number of comparisons within each hierarchy: * $p < 1x10-2$ ** $p < 5.5x10-3$

Disclosures: **M.M. Park:** None. **J.P. Lerch:** None. **A.N. Voineskos:** None. **M. Chakravarty:** None.

Nanosymposium

112. Autism: Physiology and Systems III

Location: 146C

Time: Sunday, November 16, 2014, 8:00 AM - 10:15 AM

Presentation Number: 112.04

Topic: C.06. Developmental Disorders

Support: NIH Grant NS045193

Nancy Lurie Marks Family Foundation

Simons Foundation

Title: Developmental diaschisis: A sensitive-period framework for understanding the cerebellum's role in autism

Authors: *S. S.-H. WANG, A. D. KLOTH, A. BADURA;
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Abstract: Cerebellar research has focused principally on adult motor function. However, the cerebellum also maintains abundant connections with nonmotor brain regions throughout postnatal life. Anatomical and clinical evidence in mice and humans support the view that the cerebellum guides the maturation of remote nonmotor neural circuitry. Early-life cerebellar injury is associated with gradual motor compensation but high rates of cognitive and social deficits, including autism. Perinatal cerebellar injury increases the risk of autism by approximately 40-fold, comparable to twin-level genetic inheritance. These findings are consistent with the sensitive period concept, in which early-life perturbations can have lasting consequences at remote downstream targets. Cerebellar dysfunction is likely to be associated with misprocessing of multisensory information, a common feature in early stages of autism. Recently, we have used a variety of mouse models of autism to examine a delay eyeblink conditioning, a form of cerebellar learning. We find a variety of learning and performance deficits consistent with perturbation to various components of cerebellar learning circuitry. We suggest that over development, specific cerebellar zones influence neocortical substrates for social interaction. Sensitive-period disruption of internal brain communication can account for many clinical and experimental observations. We term this concept the developmental diaschisis hypothesis of autism.

Disclosures: S.S. Wang: None. **A.D. Kloth:** None. **A. Badura:** None.

Nanosymposium

112. Autism: Physiology and Systems III

Location: 146C

Time: Sunday, November 16, 2014, 8:00 AM - 10:15 AM

Presentation Number: 112.05

Topic: C.06. Developmental Disorders

Support: NIH Grant MH092696

DOD Grant AR100276

NIH Grant HD055751

Autism Speaks Grant 4853

Title: Cortical-cerebellar abnormalities underlying visuomotor control deficits in autism spectrum disorder

Authors: *M. W. MOSCONI[1], D. E. VAILLANCOURT[2], S. A. COOMBES[2], J. A. SWEENEY[3];
[1]Univ. of Texas Southwestern Med. Ctr., Dallas, TX; [2]Univ. of Florida, Gainesville, FL; [3]Univ. of Texas Southwestern, Dallas, TX

Abstract: Sensorimotor abnormalities are common in autism spectrum disorder (ASD), and we recently demonstrated that visuomotor output is more variable in ASD. The extent to which these deficits reflect motor versus perceptual-motor impairments is not known. Further, the neural mechanisms associated with these behavioral alterations have not been studied previously in ASD. We used functional MRI to examine brain systems involved in visuomotor control in 20 individuals with ASD and 23 healthy control subjects matched on age, IQ and handedness. Participants completed a test of precision grip force in which they viewed a white FORCE bar that moved upwards with increased force toward a fixed green TARGET bar. They were instructed to press on a transducer with their index finger and thumb to maintain the FORCE bar at the level of the TARGET bar for 26 sec. We varied the gain of visual feedback, defined as the vertical distance the white bar moved per Newton of force applied to the transducer, across three levels (low, medium and high). Subjects with ASD showed increased force variability that was more severe at the lowest and highest gain levels compared to the medium gain level. Mean force levels were similar across groups. During the low gain condition, individuals with ASD showed reduced activation in contralateral primary motor and premotor cortices, bilateral anterior cerebellum (lobules I-V) and ipsilateral cerebellar lobules V/VI. When visual feedback gain was high, individuals with ASD still showed reduced activation in contralateral primary motor cortex and anterior cerebellum, but they also demonstrated increased activation in right cuneus and precuneus, supplementary motor area, middle frontal gyri, right superior temporal gyrus, right superior parietal lobule and medioposterior cerebellum. Results from the low gain condition suggest cortico-cerebellar systems are compromised in ASD when visual feedback information is degraded and individuals must rely more on non-visual feedback control processes. Findings from the high gain condition suggest that when more precise visual feedback information is available, individuals with ASD utilize visual motion processing (V3, V5) and cognitive control brain regions (middle frontal gyri, supplementary motor area) to guide force output, but these systems are not able to compensate for motor deficits and their “hyperactivity” may exacerbate motor output abnormalities. Thus, brain systems involved in controlling motor output and those involved in processing visual feedback information each are compromised in ASD and may contribute to the sensorimotor abnormalities commonly observed in this disorder.

Disclosures: M.W. Mosconi: None. **D.E. Vaillancourt:** None. **S.A. Coombes:** None. **J.A. Sweeney:** None.

Nanosymposium

112. Autism: Physiology and Systems III

Location: 146C

Time: Sunday, November 16, 2014, 8:00 AM - 10:15 AM

Presentation Number: 112.06

Topic: C.06. Developmental Disorders

Title: Striatal volumes in autism spectrum disorder (ASD)

Authors: M. SCHUETZE[1], I. CHO[1], S. VINETTE[1], M. T. M. PARK[2], M. CHAKRAVARTY[2], *S. L. BRAY[1];
[1]Univ. of Calgary, Calgary, AB, Canada; [2]Res. Imaging Ctr., Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada

Abstract: Several characteristic behaviors in individuals with ASD suggest striatal dysfunction, including repetitive motor behaviors and differences in social motivation. While some structural neuroimaging studies have found average and developmental differences in striatal volumes, there are some inconsistencies in the literature, perhaps due to smaller sample sizes. We aimed to circumvent this problem by applying volumetric analyses to anatomical MRI datasets from 15 sites (633 male subjects, 315 ASD (age range 7-35 yrs), 324 controls (age range 6.5-34.1 yrs) of the Autism Brain Imaging Data Exchange (ABIDE) project. We used a recently developed algorithm, MAGeT-Brain (Multiple Automatically Generated Templates; Chakravarty et al, 2013) for automatic segmentation of the striatum and its sub-regions. Univariate general linear models were fitted to the volume of each striatal region, including diagnosis as fixed and site as random factors, as well as age and intracranial volume (ICV) as covariates. We found a significant relative decrease of right pre-commissural putamen (p=.03), compared to the typically developing (TD) control group. Since previous research showed a nonlinear relationship between total brain volume and thalamic volume in individuals with ASD, implying an atypical development of the thalamus in ASD, we also analyzed the volume of the thalamus and its sub-regions. We found a significant relative decrease in volume of left lateral geniculate nucleus (p=.002), right medial dorsal thalamus (p=.013) and right ventral posterior nucleus (p=.017), compared to the TD control group. The p-value of left lateral geniculate nucleus stayed significant after multiple comparisons. However, p-values of the other striatal and thalamic sub-regions did not survive after accounting for multiple comparisons. We additionally tested whether there was increased inter-subject variability in volumes in the ASD group, but did not find evidence to support this hypothesis. Taken together our results suggest relatively modest differences in volume of striatal and thalamic sub-regions in ASD. Further research will be required to assess potential relationships between sub-cortical volumes and ASD symptoms in

large samples. Our study underlines the importance of data sharing projects such as ABIDE to investigate heterogenous disorders such as ASD. Furthermore, this work highlights the usefulness of automated tools such as MAGeT-Brain as a promising alternative to time-intensive manual volume segmentation for large datasets.

Disclosures: **M. Schuetze:** None. **I. Cho:** None. **S. Vinette:** None. **M.T.M. Park:** None. **M. Chakravarty:** None. **S.L. Bray:** None.

Nanosymposium

112. Autism: Physiology and Systems III

Location: 146C

Time: Sunday, November 16, 2014, 8:00 AM - 10:15 AM

Presentation Number: 112.07

Topic: C.06. Developmental Disorders

Support: NIH R01-MH081023

K01-MH097972

CDMRP AR093335

Title: White matter compromise accompanies functional overconnectivity within the imitation network in children with autism

Authors: ***M. DATKO**[1,2], I. FISHMAN[2], Y. CABRERA[2], R. CARPER[2], R.-A. MÜLLER[2]; [1]Cognitive Sci., UC San Diego, San Diego, CA; [2]San Diego State Univ., San Diego, CA

Abstract: Background: The increasing prevalence of autism spectrum disorder (ASD), a neurodevelopmental disorder characterized by sociocommunicative impairments, presents a growing public health challenge. Converging evidence indicates disrupted neural connectivity and atypical brain network organization in ASD, but it is unclear whether altered connectivity is especially prominent in brain networks that participate in social cognition. We tested for (a) altered connectivity in the imitation network, which is putatively impaired in ASD; (b) links between white matter microstructure for known pathways connecting imitation network nodes, functional connectivity of this network and clinical symptoms. **Methods**: Forty children and adolescents with ASD, ages 7-18 years, and 40 typically developing (TD) matched controls, completed resting-state functional magnetic resonance imaging (rs-fMRI) and diffusion weighted imaging scans. Whole-brain intrinsic functional connectivity (iFC) analyses were conducted using seed regions consistently activated by imitation tasks, as determined in a recent ALE meta-

analysis. Structural connectivity was analyzed using probabilistic tractography and measures derived from the diffusion tensor. Seed and target regions for tractography were derived from a subset of regions used for the FC analyses, with inclusion of nearby white matter. Robust intrahemispheric pathways consistent with known anatomy were identified between 6 region pairs in each hemisphere. DTI indices were extracted from these tracts. Head motion from functional and diffusion scans was used as a statistical covariates. **Results**: We found (a) a pattern of predominant functional overconnectivity in the imitation network in ASD, with all overconnected clusters (ASD > TD) falling outside the imitation network; (b) ASD participants with greater outside-network connectivity had greater social symptoms. Structurally, we found (a) higher radial, axial, and mean diffusivity in tracts connecting nodes of the imitation network, including inferior frontal gyrus and (pre)motor areas bilaterally; and (b) these microstructural abnormalities were also correlated with ASD social symptomatology. **Conclusions**: Our findings add to the growing evidence that functional overconnectivity in ASD is associated with symptom severity. Notably, we also found evidence of white matter compromise in tracts connecting imitation nodes (thus reflecting reduced anatomical network integration). Our study shows that multimodal investigation may reveal complementary aspects of aberrant connectivity in ASD.

Disclosures: M. Datko: None. **I. Fishman:** None. **Y. Cabrera:** None. **R. Carper:** None. **R. Müller:** None.

Nanosymposium

112. Autism: Physiology and Systems III

Location: 146C

Time: Sunday, November 16, 2014, 8:00 AM - 10:15 AM

Presentation Number: 112.08

Topic: C.06. Developmental Disorders

Support: CONICET Grant PIP2010-2012

University of Buenos Aires Grant UBACyT GEF2014-2016

ANPCyT Grant PICT2010-1334

Title: Cerebellar neuroinflammation can modulate sociability in mice

Authors: *A. M. DEPINO[1,2], L. LUCCHINA[1,2], N. KAZLAUSKAS[1,2], M. CAMPOLONGO[1,2]; [1]Inst. For Physiology, Mol. Biol. and Ne, Buenos Aires, Argentina; [2]DFBMC, FCEyN, Univ. of Buenos Aires, Buenos Aires, Argentina

Abstract: Recent clinical and experimental evidence has suggested a role of the cerebellum in the etiopathogenesis of autism spectrum disorders (ASD). A brain structure generally tasked with coordinating movements, the cerebellum has been recently proved to regulate emotions and attention. To further study the role of the cerebellum in behaviors related to autism, we used a mouse model of ASD and evaluated whether histological alterations previously described in the brain of autistic individuals, could also be observed in the model. Animals exposed to valproic acid (VPA) at the gestational day (GD) 12.5, showed in adulthood signs of microgliosis in the cerebellum and an increase in the GFAP-positive area in the granular cell layer of the lobule 7. Moreover, after a peripheral LPS stimulus, the levels of pro-inflammatory cytokines were significantly increased in the cerebella of VPA mice, when compared to control mice, suggesting a primed state of glial cells in this structure. To evaluate whether cerebellar neuroinflammation could modulate behaviors related to ASD, we studied the effect on sociability after eliciting an inflammatory response specifically in the lobule 7 of the cerebellum. Animals injected with 10ng of LPS in the lobule 6/7 of the cerebellum showed a decrease in the time spent exploring a social stimulus 24 h after injection. This effect on behavior was paralleled by an activation of microglia in the region. Interestingly, the same stimulus applied to the lobules 4/5 did not affect sociability, although the increase in microglial activation was also observed. Our results suggest a role of a specific structure within the cerebellum in the regulation of sociability in mice and contribute to recent evidences highlighting the involvement of this structure in ASD.

Disclosures: A.M. Depino: None. **L. Lucchina:** None. **N. Kazlauskas:** None. **M. Campolongo:** None.

Nanosymposium

112. Autism: Physiology and Systems III

Location: 146C

Time: Sunday, November 16, 2014, 8:00 AM - 10:15 AM

Presentation Number: 112.09

Topic: C.06. Developmental Disorders

Support: NSF IGERT Perceptual Science

Title: Peripheral noise provides a new conceptual framework for the mirror neuron systems theory

Authors: *P. YANOVICH[1], E. B. TORRES[2];
[1]Computer Sci., [2]Psychology, Rutgers Univ., Piscataway, NJ

Abstract: Humans are keen in recognizing biological motion visually, even from such a minimalistic setup as point-light displays. The Mirror Neuron System Theory (MNST) is thought to account for this ability. However, the MNST's conceptual framework examines this type of motion perception as a top-down (vision-to-action) process. This view cannot explain how the link between vision and action is developed. We have used the patterns of motor-output variability in natural motions to investigate the extent to which people have a stable kinesthetic percept and are able to discriminate the sensory-motor noise patterns inherent to their own movements from those of others. These noise patterns are a form of kinesthetic re-afference because their modulation and central control depend on the continuous returning afferent stream which those motions themselves cause. Here we present a new paradigm for studying biological motions from the kinesthetic-reafference (bottom-up) perspective during unconstrained gait. We extracted peripheral noise from unconstrained motion and used the noise patterns to add postural noise to the veridical motions captured from the same subjects. We then asked the subjects whether they could recognize themselves in the video of a computer generated character endowed with the modified motion captured data. We also captured the movement patterns of the pointing decision identifying the target. We had recently found in 6 subjects that they could systematically discriminate well above chance the peripheral motor noise patterns of continuous self-motions performed during sports routines when comparing those patterns to the patterns of others. Furthermore the stochastic signatures of kinesthetic re-afference in sports routine performance aligned well with those of the decision- making movements. We then used a new noise pattern extraction procedure that describes the transient motions rather than key postures from the previous study. We ask the extent to which we can modify a generic activity such as gait using the new procedure, before it becomes unrecognizable. We also ask if the noise patterns in decision making movements -a highly cognitively loaded motion and largely controlled by the central nervous system- are biased by the peripheral noise observed in the video. We report our results in 10 additional subjects and provide a new conceptual framework for MNST that combines both points of view: the bottom-up (action-to-vision underlying recognition of the kinesthetic self) and the top-down (vision-to-action underlying the bias in the decision movement from the visual stimuli).

Disclosures: P. Yanovich: None. **E.B. Torres:** None.

Nanosymposium

113. Traumatic Brain Injury: Exploring Mechanisms and Interventions

Location: 150B

Time: Sunday, November 16, 2014, 8:00 AM - 10:45 AM

Presentation Number: 113.01

Topic: C.10. Trauma

Support: CDMRP/DHP Grant W81XWH-12-2-0134

Title: Time course of brain vulnerability following closed-head concussion in rats: A global metabolomics analysis

Authors: *Y. D. BRYANT, L. LEUNG, R. D. READNOWER, D. A. SHEAR, F. C. TORTELLA;
Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract: An estimated 75% of all traumatic brain injury (TBI) incidences that occur each year are concussions or other forms of mild TBIs. The current study was designed to identify biochemical processes that are signature to concussive injuries in order to establish a temporal profile of these biochemical changes for potential therapeutic intervention and the assessment of therapeutic responses. Adult Sprague-Dawley rats received anesthesia (sham only), single projectile concussive impact (sPCI), or repeated PCI (rPCI; 4xPCI at 1h-interval). Ipsilateral frontal cortices were collected at 30min, 2h, 6h, 24h, 72h and 7 days post-sPCI, and at 2h post-rPCI (n=6/group/time-point). Using unbiased metabolomic profiling, we detected statistically significant differences between sham control and PCI animals. The results show alterations in neurotransmitter related metabolites in the PCI tissues, such as increased gamma-aminobutyric acid (GABA) and acetylcholine levels (vs. sham), which may influence behavior and recovery. All PCI tissues exhibited elevated levels of oxidative stress, indicated by lower glutathione and gamma-glutamyl amino acid levels compared to sham. However, a transient increase of gamma-glutamyl amino acid levels was detected in the sPCI tissues at later time points, suggestive of the restoration of redox homeostasis. Higher levels of creatine phosphate were detected in the PCI tissues vs. sham, revealing altered ATP/ADP buffering capacity in response to concussion. Elevated glucose levels were accompanied by higher levels of sorbitol and fructose in the PCI tissues (vs. sham), implicating alterations in glucose utilization and/or uptake. An imbalance in select tricarboxylic acid (TCA) cycle metabolites was observed in response to PCI, suggestive of disrupted mitochondrial metabolism. PCI tissues exhibited lower levels of polyunsaturated fatty acids that may impact eicosanoid levels, concomitant with diminished free fatty acid levels indicating a reduction in lipid oxidation. Additionally, nucleotide catabolism differed between sPCI and rPCI tissues, indicating a difference in nucleotide availability. The overall findings in this study demonstrate that the PCI leading to concussion can significantly alter the metabolomic profile of rat brain tissue. The temporal profile of this disruption revealed an early peak at 6h followed by recovery by 24h after sPCI. Furthermore, some biochemical alterations were greater following rPCI, suggesting increased brain vulnerability in response to repeated concussions.

Disclosures: Y.D. Bryant: None. **L. Leung:** None. **R.D. Readnower:** None. **D.A. Shear:** None. **F.C. Tortella:** None.

Nanosymposium

113. Traumatic Brain Injury: Exploring Mechanisms and Interventions

Location: 150B

Time: Sunday, November 16, 2014, 8:00 AM - 10:45 AM

Presentation Number: 113.02

Topic: C.10. Trauma

Title: Injury timing alters metabolic, inflammatory and functional outcomes following repeated mild traumatic brain injury

Authors: *Z. M. WEIL, K. R. GAIER, E. KARELINA;
Neurosci., Ohio State Univ. Med. Ctr., Columbus, OH

Abstract: Repeated head injuries are a major public health concern both for athletes, and members of the police and armed forces. There is ample experimental and clinical evidence that there is a period of enhanced vulnerability to subsequent injury following head trauma. Injuries that occur close together in time produce greater cognitive, histological, and behavioral impairments than do injuries separated by a longer period. Traumatic brain injuries alter cerebral glucose metabolism and the resolution of altered glucose metabolism may signal the end of the period of greater vulnerability. Here, we injured mice either once, or twice separated by three or 20 days. Repeated injuries that were separated by three days were associated with greater axonal degeneration, enhanced inflammatory responses, and poorer performance in a spatial learning and memory task. A single injury induced a transient but marked increase in local cerebral glucose utilization in the injured hippocampus and sensorimotor cortex, whereas a second injury, three days after the first, failed to induce an increase in glucose utilization at the same time point. In contrast, when the second injury occurred substantially later (20 days after the first injury), an increase in glucose utilization occurred that paralleled the increase observed following a single injury. The increased glucose utilization observed after a single injury appears to be an adaptive component of recovery, while mice with 2 injuries separated by three days were not able to mount this response, thus this second injury may have produced a significant energetic crisis such that energetic demands outstripped the ability of the damaged cells to utilize energy. These data strongly reinforce the idea that too rapid return to activity after a traumatic brain injury can induce permanent damage and disability, and that monitoring cerebral energy utilization may be a tool to determine when it is safe to return to the activity that caused the initial injury.

Disclosures: Z.M. Weil: None. **K.R. Gaier:** None. **E. Karelina:** None.

Nanosymposium

113. Traumatic Brain Injury: Exploring Mechanisms and Interventions

Location: 150B

Time: Sunday, November 16, 2014, 8:00 AM - 10:45 AM

Presentation Number: 113.03

Topic: C.10. Trauma

Support: NJCBIR Grant CBIR11PJT003

Title: Neuronal calcium regulation and function are affected by the rate of injury to the brain

Authors: A. A. ADAMS[1], G. C. MAGOU[2], J. R. BERLIN[2], *B. J. PFISTER[1];
[1]Dept Biomed Engin., New Jersey Inst. Technol., NEWARK, NJ; [2]Pharmacol. and Physiol., New Jersey Med. School, Rutgers Univ., Newark, NJ

Abstract: Rapid traumatic movements of the head can cause the brain to deform relative to the motion of the skull and in the process rapidly stretch axon fibers leading to widespread degeneration and disconnection. Here the relationship between mechanical deformation and the evolution of structural and functional alterations is studied using an *in vitro* stretch injury model. Cortical neurons cultured on an elastic silicone membrane were rapidly stretched by applying a pulse of air. Stretch injured cortical axons are known to undergo undulatory distortions, returning to original length within 20min. Stretch injury also causes a large Ca influx and sustained elevated intracellular calcium (Ca_i) that is blocked by Tetrodotoxin (TTx). Time-lapse images were used to measure the distended length (ImageJ) of the axon over 1hr post-injury $D(t_i)=L(t_i)-L_o$. 2μM fluo-4 AM ester used to measure the injury induced change in Ca_i over 120s post-injury. Fluorescence intensity was measured, background subtracted and a ratio change was calculated from pre-injury baseline $\Delta F=F(t_i)/F_o$. *Undulations*: Cultures were injured with 60% strain at strain rates of 10, 30, and $70s^{-1}$. Axons distended 15.96%±6.22 at 10 s^{-1}, 19.17%±11.5 at $30s^{-1}$, and 23.9%±15.5 at $70s^{-1}$. At 20min post injury, the percent recovery of axons to their original length was 99.96, 96.25, and 93.43% respectively. Only the slowest rate ($10s^{-1}$) was found to be significantly different from the two higher rates ($p>.05$). *Intracellular Calcium*: Cultures were stretch injured with strains of 20, 40, and 60% at strain rates of 30 and 70 s^{-1}. Oscillatory fluctuations in Ca_i were observed in the 20% strain group at both rates and for 40% at $30s^{-1}$. Stretch injury with 40% strain at $70s^{-1}$ transitioned to a sustained Ca_i without oscillations and did not return to baseline. As expected, the amount of Ca_i scaled with the amount of the applied strain. Ca influx scaled with strain rate within the 60% strain group $\Delta F=6.38\pm0.96$ at $30s^{-1}$ and 9.23±1.61 at $70s^{-1}$ but not within the 20 and 40% strain groups. Interestingly, TTx blockage of the calcium influx was lost at the higher rates of injury $\Delta F=1.17\pm0.08$ at $20s^{-1}$, 4.80±1.50 at $70s^{-1}$ and 4.81±1.12 at $100s^{-1}$. We also considered the effect of stretch injury on electrical

activity. Spontaneous action potentials recorded from injured neurons were attenuated 66% over non-injured neurons. This reduction in activity was characterized by a reduction in interval event frequency as well as reduced bursting behavior. This study establishes an important link between variations in how the brain is injured and the effect of magnitude and rate of trauma on neuronal structure and function.

Disclosures: **A.A. Adams:** None. **G.C. Magou:** None. **B.J. Pfister:** None. **J.R. Berlin:** None.

Nanosymposium

113. Traumatic Brain Injury: Exploring Mechanisms and Interventions

Location: 150B

Time: Sunday, November 16, 2014, 8:00 AM - 10:45 AM

Presentation Number: 113.04

Topic: C.10. Trauma

Support: NIH Grant GM103339

VA Merit Grant I0RX000206

VA Merit Grant I0RX000331

Title: Critical role of sphingosine in mitochondrial dysfunction after brain injury

Authors: ***T. I. GUDZ**[1,2], J. YU[1], M. KINDY[1], S. NOVGORODOV[1];
[1]Dept Neurosci., Med. Univ. South Carolina, CHARLESTON, SC; [2]Ralph H Johnson VA Med. Ctr., Charleston, SC

Abstract: In addition to immediate brain damage, traumatic brain injury (TBI) initiates a cascade of pathophysiological events producing secondary injury. The biochemical and cellular mechanisms that comprise secondary injury are not entirely understood. Herein, we report a substantial deregulation of cerebral sphingolipid metabolism in a mouse model of TBI. Sphingolipid profile analysis demonstrated increases in sphingomyelin species and sphingosine concurrently with up-regulation of intermediates of de novo sphingolipid biosynthesis in the brain. Investigation of intracellular sites of sphingosine accumulation revealed an elevation of sphingosine in mitochondria due to the activation of neutral ceramidase (NCDase) and the reduced activity of sphingosine kinase 2 (SphK2). The lack of change in gene expression suggested that post-translational mechanisms are responsible for the shift in the activities of both enzymes. Immunoprecipitation studies revealed that SphK2 is complexed with NCDase and cytochrome oxidase (COX) subunit 1 in mitochondria, and that brain injury hindered SphK2

association with the complex. Functional studies showed that sphingosine accumulation resulted in a decreased activity of COX, a rate-limiting enzyme of the mitochondrial electron transport chain. Knocking down NCDase reduced sphingosine accumulation in mitochondria and preserved COX activity after the brain injury. Also, NCDase knockdown improved brain function recovery and lessened brain contusion volume after trauma. These studies highlight a novel mechanism of secondary TBI involving a disturbance of sphingolipid-metabolizing enzymes in mitochondria and suggest a critical role for mitochondrial sphingosine in promoting brain injury after trauma.

Disclosures: **T.I. Gudz:** None. **J. Yu:** None. **M. Kindy:** None. **S. Novgorodov:** None.

Nanosymposium

113. Traumatic Brain Injury: Exploring Mechanisms and Interventions

Location: 150B

Time: Sunday, November 16, 2014, 8:00 AM - 10:45 AM

Presentation Number: 113.05

Topic: C.10. Trauma

Support: NIH R01 Grant NS36537

Title: Impaired autophagy due to lysosomal dysfunction is associated with neuronal cell death after TBI

Authors: ***C. SARKAR**[1], Z. ZHAO[1], S. AUNGST[1], B. SABIRZHANOV[1], A. I. FADEN[1], M. M. LIPINSKI[2];
[1]Shock, Trauma and Anesthesiol. Res. (STAR) Ctr., [2]Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Autophagy is a lysosome dependent major cellular degradative process. Its dysregulation has been implicated in several neurodegenerative diseases including traumatic brain injury (TBI). Although increase in markers of autophagy has been reported in brain after TBI, the cell type specificity, mechanisms and function of autophagy following TBI are still unknown. We performed a thorough analysis of autophagy after controlled cortical impact (CCI) induced brain injury in mice. We observed time dependent increase in autophagosome marker LC3-II in the cortex, which peaked at 1-3 days and then gradually decreased by day 7 following injury. This was confirmed by fluorescent image analysis for autophagosome marker LC3 in cortical tissue sections of sham and injured transgenic mice expressing GFP-tagged LC3. Autophagosome accumulation predominantly occurred within neurons at day 1 after TBI and in

microglia and oligodendrocytes at day 3 and day 7, respectively. Increased accumulation of autophagosomes at days 1-3 after TBI was accompanied by markedly higher level of autophagic substrate p62/SQSTM1 in the cortex, suggesting impairment of autophagosome degradation. Block of autophagic flux was confirmed by ex vivo experiment using injured and control mouse brain slices treated with inhibitor of lysosomal function, chloroquine. Thus these data clearly demonstrate that accumulation of autophagosomes after TBI is caused due to block of autophagosome clearance. This early impairment of autophagy is at least in part because of lysosomal dysfunction, as evidenced by lower protein levels and enzymatic activity of lysosomal enzyme, cathepsin D in injured cortex at day 1 after TBI. Furthermore, we observed co-localization of both caspase-dependent (cleaved caspase-3 and caspase-12) and -independent (AIF) cell death markers with GFP-LC3 signal in many cells around the site of injury at early time points (1-3 days) following TBI. Taken together our results clearly suggest that the defect in autophagy flux may contribute to neuronal cell death after TBI. Therefore we propose that restoration of lysosomal function early after TBI may restore autophagosome clearance and would provide an effective therapeutic strategy to limit neuronal loss following TBI.

Disclosures: C. Sarkar: None. **Z. Zhao:** None. **S. Aungst:** None. **B. Sabirzhanov:** None. **A.I. Faden:** None. **M.M. Lipinski:** None.

Nanosymposium

113. Traumatic Brain Injury: Exploring Mechanisms and Interventions

Location: 150B

Time: Sunday, November 16, 2014, 8:00 AM - 10:45 AM

Presentation Number: 113.06

Topic: C.10. Trauma

Support: Operation Reentry North Carolina

Title: Circulating miRNA as biomarkers for mild traumatic brain injury: implications in neurological regulations

Authors: *D. L. DOBBINS, X. PAN;
East Carolina Univ., Greenville, NC

Abstract: Although usually undiagnosed and untreated, mild traumatic brain injuries (mTBIs) are prevalent especially in military personnel and often result in neurological deficits including cognitive and emotional impairments. Severe traumatic brain injuries are detectable with modern imaging techniques, but mTBIs often lack proper diagnosis. This project aims to profile blood

miRNA expression following mild brain injury and to select biomarker candidates for clinical diagnosis. First an mTBI rodent model was generated by subjecting Sprague-Dawley (SD) rats to blast overpressure of 10-12 psi produced from a blasting simulator using compressed air. Histological and subtle biochemical changes were observed compared to sham controls. Total RNAs were extracted from whole blood using an optimized method. Aberrantly expressed miRNAs were characterized using microarray followed by quantitative real-time PCR (qRT-PCR). A suite of miRNAs were found aberrantly expressed in blast-exposed rats, which target various neurological pathways including inflammation (such as chemokine signaling pathway, cytokine-cytokine interaction pathway etc.), neuroactive ligand and receptor interactions, and neurotrophin signaling pathways, etc. Further analysis using morris water maze testing revealed cognitive and memory deficits, known effects of mTBI subjects. This study shows the potential of several conserved miRNAs to be implemented as non-invasive biomarkers of mTBIs. The dynamic changes of miRNA markers may further indicate prolonged effects and recovery progression following mTBIs.

Disclosures: D.L. Dobbins: None. **X. Pan:** None.

Nanosymposium

113. Traumatic Brain Injury: Exploring Mechanisms and Interventions

Location: 150B

Time: Sunday, November 16, 2014, 8:00 AM - 10:45 AM

Presentation Number: 113.07

Topic: C.10. Trauma

Support: Neural Injury and Plasticity Grant T32NS041218

NIH Diversity Supplement R01 NS081068-01A1S1

Title: The influence of APOE genotype on dendritic spine levels following mild TBI

Authors: *C. WINSTON, M. PARASADANIAN, D. J. BARTON, A. NEUSTADTL, D. ZAPPLE, M. P. BURNS;
Neurosci., Georgetown Univ., Washington, DC

Abstract: Dendritic spine loss is an early consequence of traumatic brain injury (TBI). Spine loss could potentially explain why patients report a variety of symptoms after injury. Genetic predisposition has also been shown to influence severity and recovery following TBI. The apoE4 allele is synonymous with poorer recovery and death after TBI; the incidence of this gene is

increased in those who suffer from Chronic Traumatic Encephalopathy (CTE); and a growing number of studies have associated the detrimental effects of apoE4 with facilitating a more pro-inflammatory state in the brain. The mechanism by which apoE isoforms differentially influence recovery and inflammatory status is not well understood. Here, we wanted to determine the role of APOE genotype on dendritic spine levels and inflammation, following single and repeat mTBI. We administered a midline, close-head impact to adult APOE3 and APOE4 targeted-replacement (TR) mice and visualized neurons and dendritic spines 24h post injury using Golgi stain. All mice present with no evidence of cell loss or neuroinflammation after a single mTBI; however mTBI caused a 12.4% decrease in dendritic spine number on apical oblique (AO) dendrites in layer II/III of injured APOE3 mice. In contrast, mTBI caused a 15.4% increase dendritic spine number on AO dendrites in layer II/III of injured APOE4 mice. After repeat mTBI (single injury, 30 days), injured APOE4 mice still presented with elevated spine levels while spine levels returned to baseline in injured APOE3 mice. We also found that injured APOE3 mice had an average reflex return time of 105s following single and repeat mTBI, while sham APOE3 mice had an average reflex return time of 51s. Interestingly, injured APOE4 mice had an average reflex return time of 71s after single mTBI, however following repeat mTBI, average reflex return time wasn't significantly different from sham APOE mice. Injured APOE4 mice displayed more white matter inflammation and damage of the optic tract, compared to injured APOE3 mice, which persisted up to two months following the final impact. Here, our findings demonstrate that APOE genotype differentially influences dendritic spine levels, reflex return time, and promotes a pro-inflammatory state in the brain following mTBI.

Disclosures: C. Winston: None. **M. Parasadanian:** None. **D.J. Barton:** None. **A. Neustadtl:** None. **D. Zapple:** None. **M.P. Burns:** None.

Nanosymposium

113. Traumatic Brain Injury: Exploring Mechanisms and Interventions

Location: 150B

Time: Sunday, November 16, 2014, 8:00 AM - 10:45 AM

Presentation Number: 113.08

Topic: C.10. Trauma

Support: NIH Grant R01HD059288

Title: *In vivo* hippocampal neuronal oscillations are altered after traumatic brain injury and restored by dietary therapy

Authors: *R. PATERNÒ[1], B. JOHNSON[1], J. ELKIND[1], C. SMITH[1], G. XION[1], A. COHEN[1,2]; [1]Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Cognitive impairment caused by traumatic brain injury (TBI) can lead to devastating consequences for both patients and their families. The underlying neurological bases for TBI-induced cognitive dysfunction remain unknown. However, many lines of research have implicated the hippocampus in the pathophysiology of traumatic brain injury. In particular, past research has found that theta oscillations, long thought to be the electrophysiological basis of learning and memory, are decreased in the hippocampus following TBI. Here, we recorded in vivo electrophysiological activity in the hippocampi of fourteen mice, seven of which had previously been exposed to traumatic brain injury. Consistent with previous data, we found that theta oscillations in hippocampus were decreased in the TBI animals compared to sham control animals. Furthermore, we also determined that power in the slow gamma band was also decreased in TBI mice. Finally, we administered dietary therapy consisting of branched chain amino acids (BCAAs) leucine, isoleucine and valine initiated 48 hours after the injury and maintained for 5 days in four brain injured mice, and found that theta oscillations returned to healthy levels after dietary treatment. Thus, these data represent the first evidence that both theta and gamma oscillations are specifically diminished in brain injured mice, and provide proof of concept that dietary supplementation can restore oscillatory function and, potentially, normal cognition after brain injury.

Disclosures: R. Paternò: None. **B. Johnson:** None. **J. Elkind:** None. **C. Smith:** None. **G. Xion:** None. **A. Cohen:** None.

Nanosymposium

113. Traumatic Brain Injury: Exploring Mechanisms and Interventions

Location: 150B

Time: Sunday, November 16, 2014, 8:00 AM - 10:45 AM

Presentation Number: 113.09

Topic: C.10. Trauma

Support: NIH/NINDS NRSA 1F31NS083243

NIH Grant 5R01HD059288-10

Title: Layer specific alterations in synaptic transmission in prefrontal cortex following mild traumatic brain injury

Authors: *C. SMITH[1,2], G. XIONG[1], J. ELKIND[1], C. CRUZ[1], C. PALMER[1,2], A. COHEN[1]; [1]Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: More than 1% of Americans suffer from a Traumatic Brain Injury each year, and even mild Traumatic Brain Injury (mTBI) can cause long-lasting neurological effects (Langlois et al., 2006). Despite its prevalence, currently no therapy exists to mitigate or treat the underlying causes of cognitive impairments suffered by mTBI patients. In order to model mTBI in mice we employed lateral fluid percussion injury (LFPI). LFPI is a routinely employed rodent model of brain injury that reproduces key features of human TBI including neuronal cell loss, gliosis, ionic perturbation and memory deficits (Dixon et al., 1987, McIntosh et al., 1987, 1989, Smith et al., 1991). LFPI is known to alter the balance of excitatory/inhibitory synaptic transmission in the hippocampus, disrupting both circuit function and behavior (Witgen et al., 2005, Cole et al., 2010). Following an LFPI designed to mimic an mTBI we investigated alterations in synaptic transmission in the prelimbic cortex, the functional homolog of human mPFC. Amplitude of field excitatory postsynaptic potentials recorded in layer 5 was reduced in brain-injured mice. Furthermore, spontaneous and miniature excitatory synaptic currents onto layer 2/3 cells were more frequent in slices derived from LFPI mice. Inhibitory currents onto layer 2/3 cells were slightly smaller in LFPI slices. Additionally, an increase in action potential threshold was observed in layer 2/3 cells, possibly in response to the shift in excitatory/inhibitory balance. Conversely, no differences in excitatory or inhibitory synaptic transmission onto layer 5 cells were observed. Ongoing investigations attempt to link these synaptic alterations to working memory deficits. These results demonstrate that both excitatory and inhibitory synaptic transmission in layer 2/3, but not layer 5 of the mPFC are altered by mTBI and may contribute to observed behavioral and cognitive deficits.

Disclosures: C. Smith: None. **G. Xiong:** None. **J. Elkind:** None. **C. Cruz:** None. **C. Palmer:** None. **A. Cohen:** None.

Nanosymposium

113. Traumatic Brain Injury: Exploring Mechanisms and Interventions

Location: 150B

Time: Sunday, November 16, 2014, 8:00 AM - 10:45 AM

Presentation Number: 113.10

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Swedish medical research Council Grant Nr 2710

US air Force Material Command grant number FA8655-05-1-3065

India-EU Grant Nr.456-70-32-11

Ministry of Science & Technology, Govt. of India DST-DBT-56-23155.69

Title: Nanowired Cerebrolysin potentiates mesenchymal stem cells induced neuroprotection and neurorepair following concussive head injury

Authors: *H. S. SHARMA[1], D. F. MURESANU[2], H. MOESSLER[3], A. SHARMA[4];
[1]Uppsala Univ., Uppsala, Sweden; [2]Clin. Neurosciences, Univ. of Med. & Pharm., Cluj-Napoca, Romania; [3]Ever Neuro Pharma, Oberburgau, Austria; [4]surgical Sciences, Anesthesiol. & Intensive Care Med., Uppsala Univ. Hosp., Uppsala, Sweden

Abstract: Concussive head injury (CHI) could lead to either instant death or lifetime disabilities. Our soldiers are highly vulnerable to CHI during combat operations. Thus, to treat these soldiers effectively novel strategies using combination therapy is needed. Since stem cell therapy enhances neurorepair in brain or spinal cord injuries, this is quite likely that this may be effective in CHI as well. However, this is still unclear whether CHI occurring at high environmental temperature could adversely affect the outcome with regard to brain pathology or sensory motor disturbances. Since our soldiers are often engaged in combat operations in desert environments under high heat conditions, this is likely that their outcome following CHI require special treatment using combination therapy. In this investigation we used nanowired delivery of mesenchymal stem cells (MSCs) intravenously following CHI and also added a known neuroprotective multimodal drug Cerebrolsyin with or without nanowired delivery to see whether a synergistic better effects of this combination can result in good neurorepair following CHI at high environmental heat situations. CHI was inflicted in our rat model using a weight drop of 114.6 g on the parietal skull bone under Equithesin anesthesia from a 20 cm height using a guide tube. This arrangement induces an impact of 0.224 N on the right parietal skull surface causing serious brain edema and volume swelling particularly in the left hemisphere due to a "counter-coup" phenomenon see at 48 h after the insult. When rats were exposed at 38°C for 1 h daily until 2 weeks and then the identical CHI was delivered, the magnitude and intensity of brain edema development and volume swelling was exacerbated by 2 to 3 fold as compared to the CHI delivered in rats kept at room temperature (21±1°C). Commercially available MSCs (1 million cells) were delivered in a group of rats with CHI either at room temperature or in heat treated animals resulted in a mild but significant reduction in volume swelling and brain edema formation. However, when TiO2 nanowired MSCs are given under identical conditions CHI did not results in massive brain edema formation or volume swelling at room temperature. However, heat stressed rats did not show sufficient reduction in brain pathology. Interestingly when TiO2 nanowired Cerebrolsyin (2.5 ml/kg) was co-administered with nanowired MSCs either 8 or 12 h after CHI significant reduction in brain pathology and brain edema formation was seen in heat stressed rats at 48 h. These observations are the first to demonstrate that a combination of nanowired Cerebrolsyin and MSCs synergistically induced efficient neurorepair, not reported earlier.

Disclosures: **H.S. Sharma:** None. **D.F. Muresanu:** None. **H. Moessler:** None. **A. Sharma:** None.

Nanosymposium

113. Traumatic Brain Injury: Exploring Mechanisms and Interventions

Location: 150B

Time: Sunday, November 16, 2014, 8:00 AM - 10:45 AM

Presentation Number: 113.11

Topic: C.10. Trauma

Support: NIH R01 CA133216

NIH R21 AG042016

Alzheimer's Association IIRG-11-202064

Title: CX3CR1 deficiency ameliorates TBI-induced inflammatory response and cognitive dysfunction

Authors: ***J. M. MORGANTI**, T. D. JOPSON, L. K. RIPARIP, S. ROSI;
Brain and Spinal Injury Ctr., Univ. of California San Francisco, San Francisco, CA

Abstract: The exact role of neuron-microglia communication through CX3CL1-CX3CR1 signaling in neurodegenerative disorders remains elusive as recent studies targeting this pathway have shown both neuroprotective and neurotoxic properties. Traumatic brain injury (TBI) initiates a robust activation of microglia, which has been shown to persist for years following the initial event, and can ultimately result in neurodegeneration. In the current study we examined the effect of CX3CR1 deletion (*CX3CR1*$^{GFP/GFP}$) upon multiple pathways underlying TBI-induced neurotoxic responses both at acute (24hrs) and chronic (3 months) time points after injury. TBI or sham surgery was induced by controlled cortical impact in *CX3CR1*$^{GFP/GFP}$ mice (KO) and wild type (WT) animals. 24 hrs following TBI, KO animals had a reduced neuroinflammatory response compared to WT mice. Specifically, KO mice had significantly decreased expression of the pro-inflammatory mediators *IL-1β, TNFα, NOS2, and IL6* compared to WT-TBI mice. We next examined the effect of CX3CR1 deletion upon TBI-induced hippocampal-dependent cognitive function 3 months after injury using the radial arm water maze (RAWM). Although KO mice had a higher baseline for errors during day one of RAWM, they had a significantly ameliorated response (decreased errors) compared to WT-TBI mice. Isolated hippocampi from these animals were analyzed for multiple markers associated with synaptic

function by Western blot analyses. Our results demonstrate that TBI alters post-synaptic NMDAr, as the NR2b- but not the NR2a subunit was significantly increased in WT-TBI mice, however this effect was abrogated in KO-TBI mice. Furthermore, TBI induced a significant increase in the Src-like kinase Fyn as well as the phosphorylation of p44/42 MAP kinase in WT mice, which again was abrogated in KO-TBI mice. Interestingly, we did observe a strong trend for increased PSD-95 in WT-TBI mice compared to sham, which was blunted in KO-TBI mice. Additionally, we observed similar trends for increased phosphorylated tau in WT-TBI animals. Taken together, these data indicate that CX3CR1 deletion prevents the TBI-induced pro-inflammatory and neurotoxic response acutely, which may in part underlie the ameliorated response of TBI-induced synaptic dysfunction chronically.

Disclosures: **J.M. Morganti:** None. **T.D. Jopson:** None. **L.K. Riparip:** None. **S. Rosi:** None.

Nanosymposium

114. Gene Therapy

Location: 140A

Time: Sunday, November 16, 2014, 8:00 AM - 10:15 AM

Presentation Number: 114.01

Topic: C.14. Gene Therapy

Support: Advanced ERC Grant

Title: Site specific labelling of AAV9 to investigate viral entry into the central nervous system

Authors: ***J. CHANDRAN**, P. SHARP, M. AZZOUZ;
Sheffield Inst. for Translational Neurosci., Univ. of Sheffield, Sheffield, United Kingdom

Abstract: A major bottleneck to therapeutics targeting neurological disorders is the blood-brain barrier (BBB), which restricts potentially harmful chemicals from entering the brain, while regulating transport of essential molecules from the circulatory system. A promising method for delivering molecules across the BBB is to use adeno-associated vectors (AAVs), which for some serotypes (AAV9) can transduce the central nervous system (CNS) without invasive surgery, making these promising candidates for CNS gene therapy applications. Currently, however the mechanism by which they cross the BBB and transduce cells in the CNS is unclear. To address this limitation, we have inserted a 12 amino acid sequence into the viral capsid that is capable of a highly specific fluorescently-conjugated thiol-selective modification, which allows us to track individual viral particles in vitro and in vivo, without affecting viral packaging and titer. We have used this technique to image in real time, viral entry across an in vitro BBB model

consisting of brain endothelial cells and astrocytes separated by a porous membrane. We have also tracked the viral particles in vivo, using intravital imaging through an optically clear skull in a live, anesthetized mouse to examine viral entry across the blood brain barrier, and have delineated a time course of viral entry into the CNS. We expect our data will provide key spatiotemporal data regarding viral entry of the AAV9 vector into the brain, and clarify the mechanism by which the AAV9 virus crosses the BBB, which is important given the growing use of this viral vector in a number of human clinical trials.

Disclosures: J. Chandran: None. **P. Sharp:** None. **M. Azzouz:** None.

Nanosymposium

114. Gene Therapy

Location: 140A

Time: Sunday, November 16, 2014, 8:00 AM - 10:15 AM

Presentation Number: 114.02

Topic: C.14. Gene Therapy

Support: MDA 216676

PO1 HL59412-06

1R01HD052682-01A1

Title: Gene therapy improves motor function in early to moderate stages of Pompe disease

Authors: *D. J. FALK[1], A. G. TODD[1], G. A. WALTER[2], S. LEE[3], L. NOTTERPEK[3], D. D. FULLER[4], B. J. BYRNE[1];
[1]Pediatrics, [2]Physiol. and Functional Genomics, [3]Neurosci., [4]Physical Therapy, Univ. of Florida, Gainesville, FL

Abstract: Pompe disease is a glycogen storage disorder caused by loss of acid-alpha glucosidase (GAA) and characterized by the systemic accumulation of lysosomal glycogen. One of the earliest detectable pathological hallmarks is disruption to the neuromuscular junction indicating glycogen-associated pathology within the motor neuron and muscle. We have recently characterized a temporal pattern of neuromuscular synaptic pathology associated with Pompe disease as depicted by increased size and fragmentation of the motor endplate, significant reduction in the levels of neurofilament proteins, alterations in axonal fiber diameter and myelin thickness within the sciatic and phrenic nerves. Marked changes in the mRNA acetylcholine receptor expression profile in *Gaa-/-* mice were also observed during early and advanced stages

of disease reflecting incomplete innervation at the neuromuscular junction. AAV9 vector administration to replace GAA in both skeletal muscle and the motor neuron reveals a temporal pattern where therapeutic efficacy is limited to restore molecular, histological and functional parameters. While AAV9 was sufficient to improve motor function in early and moderate stages, AAV9 therapy was insufficient to restore functional performance within animals treated with advanced-stage disease despite clearance of glycogen from the treated muscle. Taken together our results indicate the neuromuscular junction is a primary pathological target in Pompe disease that is critical for proper respiratory and motor function.

Disclosures: **D.J. Falk:** None. **A.G. Todd:** None. **G.A. Walter:** None. **S. Lee:** None. **L. Notterpek:** None. **D.D. Fuller:** None. **B.J. Byrne:** None.

Nanosymposium

114. Gene Therapy

Location: 140A

Time: Sunday, November 16, 2014, 8:00 AM - 10:15 AM

Presentation Number: 114.03

Topic: C.14. Gene Therapy

Support: European Research Council

Title: Design of AAV-mediated CNS-targeted gene delivery system using neuronal promoters

Authors: ***V. LUKASHCHUK**, I. COLDICOTT, P. J. MULCAHY, B. MUSZYNSKI, K. NING, M. AZZOUZ;
Univ. of Sheffield, Sheffield, United Kingdom

Abstract: One of the key challenges in treating inherited forms of neurodegenerative disorders such as motor neuron disease is targeted delivery of the therapeutic genes to motor neurons. While overexpression or knocking down certain genes in the affected tissue of the patient may be beneficial for improving the symptoms and the survival, the outcome of disseminated expression of those genes in other tissues and potential long-term side effects cannot be predicted. It is therefore important to design a gene therapy approach that would (a) target the gene expression to the brain and spinal cord; (b) provide sustained gene expression levels; (c) ensure that the means of delivery is minimally invasive and non-pathogenic to the host immune system. To satisfy these requirements, one of the chosen candidate promoters to target CNS in this study was motor-neuron specific Hb9. In our system we have used self-complementary recombinant adeno-associated virus of serotype 9 (scAAV9) that contains short enhancer sequences of Hb9 fused to

cytomegalovirus (CMV) minimal promoter driving the expression of GFP reporter. scAAV9 virus stocks were produced to high titers, and used to transduce spinal cord cultures from E13 mouse embryo for *in vitro* analysis and specificity of gene expression. We observed abundant GFP expression in neuronal cells of the spinal cord culture, including motor neurons, but not in glial cells, suggesting the feasibility of such an approach for selective targeting of the spinal cord neurons *in vitro*. Ongoing *in vivo* neonatal mouse gene transfer experiments are aimed at establishing whether such vector system could be efficiently applied for targeted delivery of the therapeutic genes in the mouse model of spinal muscular atrophy and amyotrophic lateral sclerosis.

Disclosures: V. Lukashchuk: None. **I. Coldicott:** None. **P.J. Mulcahy:** None. **B. Muszynski:** None. **K. Ning:** None. **M. Azzouz:** None.

Nanosymposium

114. Gene Therapy

Location: 140A

Time: Sunday, November 16, 2014, 8:00 AM - 10:15 AM

Presentation Number: 114.04

Topic: C.14. Gene Therapy

Support: Leblang Charitable Foundation

NINDS R21NS081374-01

Title: Adeno-associated virus serotype 9-mediated gene therapy for x-linked adrenoleukodystrophy

Authors: Y. GONG[1], C. A. MAGUIRE[1], D. MU[1], A. MOSER[2], J. REN[1], *F. EICHLER[3]; [1]Massachusetts Gen. Hosp. | Harvard Med. Sch., Charlestown, MA; [2]Kennedy Krieger Inst. / Johns Hopkins, Baltimore, MA; [3]Massachusetts Gen. Hosptial | Harvard Med. Sch., Charlestown, MA

Abstract: X-linked adrenoleukodystrophy (X-ALD) is a devastating neurological disorder caused by mutations in the ABCD1 gene that encodes a peroxisomal ATP-binding cassette transporter (ABCD1 aka ALDP, ALD protein) responsible for transport of CoA-activated very long chain fatty acids from the cytosol into the peroxisome for degradation. Recently, human trials of AAV-mediated gene delivery for neurological diseases have shown promise. In the present study, we used AAV9 vector delivery of the ABCD1 gene to mouse central nervous

system (CNS) to assess and optimize gene correction in X-ALD. AAV9 encoding human ABCD1(AAV9-hABCD1) was applied in vitro and in vivo. Primary cultured brain glia cell mixture from ABCD1-/- mice was transduced with different doses of AAV9-hABCD1 (or AAV9-GFP for transduction efficiency quantification and control) and subsequently cells were collected for western blot, immunofluorescence and lipid analysis. In vivo, AAV9-hABCD1 was delivered to ABCD1-/- mouse CNS by stereotactic intraventricular (ICV) injection and intravenous (IV). Tissues were harvested for gene expression, protein expression and lipid analysis. Efficient delivery of hABCD1 gene was achieved by AAV9 both in vitro and in vivo as measured by GFP expression with the control vector as well immunofluorescence detection of hABCD1. Astrocytes, microglia and neurons were the major target cell types following ICV injection while IV injection delivered to these cell types as well as microvascular endothelial cells and oligodendrocytes. Immunofluorescence showed co-staining of ALDP and catalase, indicating localization of ALDP to the peroxisome. AAV9-hABCD1 showed a dose dependent effect in reducing very long chain fatty acid (C26:0 LPC, lyso-phosphatidylcholine) levels in ABCD1-/- mouse brain cell culture, while IV injection of 1E12gc/mouse also reduced C26:0 LPC in ABCD1-/- mouse brain and spinal cord. We conclude that AAV9-mediated ABCD1 gene transfer is able to reach target cells in the nervous system as well as reduce very long chain fatty acids in culture and a mouse model of X-ALD.

Disclosures: Y. Gong: None. **C.A. Maguire:** None. **D. Mu:** None. **A. Moser:** None. **J. Ren:** None. **F. Eichler:** None.

Nanosymposium

114. Gene Therapy

Location: 140A

Time: Sunday, November 16, 2014, 8:00 AM - 10:15 AM

Presentation Number: 114.05

Topic: C.14. Gene Therapy

Support: Irish Cancer Society PCI11ODR

Title: Receptor-mediated siRNA delivery to the central nervous system with surface functionalized cyclodextrin nanoparticles

Authors: M. MALHOTRA[1], D. J. MCCARTHY[1], M. GOODING[2], B. M. D. C. GODINHO[1], R. DARCY[2], *J. CRYAN[1], C. M. O'DRISCOLL[1];
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Abstract: Non-viral nanoparticles have emerged as potential delivery vehicles for nucleic acids and drugs. Nanoparticles developed from cyclodextrins have been explored for siRNA delivery in neuronal cells. Cyclodextrins are cyclic non-reducible oligosaccharides that consist of glucopyranose units linked together via α-(1-4) glycoside bonds. Their amphiphilic nature makes them an ideal nanocarrier for delivery of hydrophobic drugs and in addition be modified with various functional groups due to the presence of hydroxyls on their primary and secondary faces. The current work focuses on the synthesis and development of amphiphilic cyclodextrin nanoparticles, which was co-formulated with other amphiphilic cyclodextrin derivatives, comprising of a hydrophilic polymer, PEG and a targeting peptide of 29 amino acids, rabies virus glycoprotein (RVG). The developed nanoparticles were characterized for their size, surface charge, siRNA loading capacity, stability and were investigated to deliver a functional siRNA against a target housekeeping gene (GAPDH) in human glioblastoma cells (U87). Our results indicate the development of stable, surface modified, targeted, cyclodextrin nanoparticles with a size of 281 ± 39.72 nm and a positive surface charge of 26.73 ± 3 mV, which showed efficient cellular uptake and a 27.24% gene-knockdown ability. Our ongoing studies include in-vitro investigations on RVG-tagged cyclodextrin nanoparticles to cross the blood brain barrier and eventually be used in-vivo for systemic delivery of siRNA, targeting brain disorders such as Huntington's Disease and ALS.

Disclosures: M. Malhotra: None. **M. Gooding:** None. **R. Darcy:** None. **J. Cryan:** None. **C.M. O'Driscoll:** None. **D.J. McCarthy:** None. **B.M.D.C. Godinho:** None.

Nanosymposium

114. Gene Therapy

Location: 140A

Time: Sunday, November 16, 2014, 8:00 AM - 10:15 AM

Presentation Number: 114.06

Topic: C.14. Gene Therapy

Support: Wings for Life

Nielsen Foundation

University of Kentucky Viral Production Core

University of Kentucky Flow Cytometry Core

Title: Promoting a targeted neuroprotective immune response

Authors: *W. M. BAILEY[1], K. D. FOUST[2], J. B. FOSTER[2], J. C. GENSEL[1];
[1]Spinal Cord and Brain Injury Res. Ctr., Univ. of Kentucky, Lexington, KY; [2]Dept. of Neurosci., The Ohio State Univ. Wexner Med. Ctr., Columbus, OH

Abstract: Macrophages, derived from resident microglia and blood monocytes, persist indefinitely at sites of spinal cord injury (SCI) and contribute to both pathological and reparative processes. More specifically, the classically activated macrophage phenotype (M1) is associated with cell loss and pathology whereas the alternatively activated phenotype (M2) may promote cell protection, regeneration, and axon plasticity in response to injury. Unfortunately, the post-injury environment drives macrophages toward an M1 phenotype. Therefore driving and sustaining an M2 phenotype would involve either changing the environment or the way cells respond to the environment. Focusing on the later approach, our goal is to develop and refine methodology for genetically engineering macrophages to maintain an M2 phenotype in the injured CNS. While preliminary data demonstrate that transplanted primary macrophages transduced ex-vivo to overexpress M2-associated genes retain an M2 phenotype in injured spinal cord, this technique has limitations due to the variability in transduction efficiency and the stability of isolated cells. We are optimizing a method to directly target microglia / macrophages in vivo using a cell-specific promoter in a viral vector. In order to identify the optimal promoter the transduction efficiency and specificity of candidate promoter regions are being investigated with qPCR, flow cytometry, and immuno-staining. Once developed, this targeted approach will be a powerful tool to analyze the role of M2 phenotype macrophages in the dynamics of progression and repair in spinal cord injury. Further, this could lead to a clinical therapy relevant to the broad spectrum of injuries in which microglia / macrophages are involved.

Disclosures: W.M. Bailey: None. **K.D. Foust:** None. **J.C. Gensel:** None. **J.B. Foster:** None.

Nanosymposium

114. Gene Therapy

Location: 140A

Time: Sunday, November 16, 2014, 8:00 AM - 10:15 AM

Presentation Number: 114.07

Topic: C.14. Gene Therapy

Support: CNPq

CAPES

DECIT/MS

FAPERJ

Title: Gene and cell therapy: Two approachs to promote neuroprotection and neuroregeneration in a model of CNS injury

Authors: *G. NASCIMENTO DOS SANTOS, L. TEIXEIRA-PINHEIRO, A. SILVA-JUNIOR, R. MENDEZ-OTERO, H. PETRS-SILVA, M. SANTIAGO;
Inst. de Biofísica Carlos Chagas Filho - IBCCF, Univ. Federal Do Rio De Janeiro, Rio De Janeiro, Brazil

Abstract: After injury, the axons in the central nervous system (CNS) are not able to regenerate great distances and permanently lose their connections. Two promising approaches to reverse this condition are cell and gene therapies. In previous work, we demonstrated that the intravitreous transplantation of bone marrow mononuclear cells (BMMC) or mesenchymal stromal cells (MSC) are capable of promoting an increase in retinal ganglion cells (RGCs) survival and axonal outgrowth. In the present work, we evaluated the therapeutic potential of pigment epithelium derived factor (PEDF) gene therapy using adeno-associated viral vectors (AAV) in a model of rat optic nerve crush. Adult (3-4 months old) Lister rats underwent unilateral optic nerve crush 30 days after AAV-PEDF, AAV-GFP (for AAV infection/transduction control) or vehicle injection into the vitreous body. Twenty eight days after injury, RGC survival was evaluated assessing the number of Brn3a-positive cells in flat-mounted retinas. PEDF gene therapy through adeno-associated viral vectors significantly increase the fraction (test/control) of Brn3a-positive cells in the retina (0.67 ± 0.05) when compared to AAV-GFP (0.39 ± 0.07) or vehicle (0.32 ± 0.09). The axonal regeneration of the surviving RGCs will be further evaluated. Considering the data previously obtained by our group showing that cell therapy with MSC or BMMC promotes neuroprotection and neuroregeneration of RGC, we intend to combine gene and cell therapies in order to enhance the beneficial effects we have obtained so far.

Disclosures: **G. Nascimento Dos Santos:** None. **L. Teixeira-Pinheiro:** None. **A. Silva-Junior:** None. **R. Mendez-Otero:** None. **H. Petrs-Silva:** None. **M. Santiago:** None.

Nanosymposium

114. Gene Therapy

Location: 140A

Time: Sunday, November 16, 2014, 8:00 AM - 10:15 AM

Presentation Number: 114.08

Topic: C.14. Gene Therapy

Support: NINDS

Title: AAV-based neonatal gene therapy in the hyperargininemic mouse prevents cortical dendritic atrophy and stimulates neuronal maturation

Authors: *R. F. MERVIS[1,2], S. K. FOLEY[1,3], N. BHATIA[3], S. PATEL[3], A. PENA[3], N. MUTHAVARAPU[3], M. DANG[3], G. C. NIETO[4], C. HU[4], G. S. LIPSHUTZ[4,5,6];
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Abstract: Arginase deficiency is a rare autosomal recessive metabolic disorder resulting from a loss of arginase I (ARG 1), the final enzyme in the urea cycle which detoxifies ammonia in mammals. The deficiency is characterized by hyperargininemia and infrequent episodes of hyperammonemia. Human patients exhibit neurological impairment with severe mental and growth retardation. In the murine model, the Arg1-/- phenotype results in neurodevelopmental issues (microcephaly, seizures) and is lethal, with death occurring by postnatal day 17. Previously, using the mouse model, it has been shown that adeno-associated virus (AAV) neonatal gene therapy for arginase deficiency resulted in normal cognitive development and long term survival. The goal of this study was to characterize the effects of the AAV-based gene therapy on developing brain circuitry in the murine model as seen in changes in the cortical dendritic arbor. Arg-1 deficient mice were generated by replacing exon 4 of the Arg1 gene with the neomycin resistance gene. AAV-based gene therapy was initiated on postnatal day 2. Controls were heterozygous littermates. Subjects were sacrificed at 14 and 28 days-old. There were three 14do mice groups: controls, Arg1 KO mice, and AAV-treated Arg1 KO mice. There were two 28do groups: controls and AAv-treated Arg1 KO mice. (Untreated Arg1 KO mice do not survive). Formalin-fixed brain blocks from the 14 and 28do groups were Golgi impregnated and coded slides prepared for morphometric analysis of dendritic branching of layer V pyramids of the parietal cortex. In 14do mice, dendritic branching of the untreated Arg KO mice was significantly less than (-18%), and the AAV-treated Arg KO mice significantly more than (+10%), the controls. In the 28do mice, both controls and AAV-treated Arg KO mice had equivalent amounts and complexity of the dendritic arbor. The results suggest that in the murine model neonatal AAV-based gene therapy expressing arginase is effective in not only preventing neurological dysfunction, but promotes the development of normal brain circuitry. Similar AAV-based gene therapies may be beneficial in the treatment of related neurodegenerative disorders.

Disclosures: **R.F. Mervis:** None. **S.K. Foley:** None. **N. Bhatia:** None. **S. Patel:** None. **A. Pena:** None. **N. Muthavarapu:** None. **M. Dang:** None. **G.S. Lipshutz:** None. **C. Hu:** None. **G.C. Nieto:** None.

Nanosymposium

114. Gene Therapy

Location: 140A

Time: Sunday, November 16, 2014, 8:00 AM - 10:15 AM

Presentation Number: 114.09

Topic: C.14. Gene Therapy

Support: NIH Grant DA030177

NIH Grant MH077995

NIH Grant AA016194

NIH Grant RR00168

Title: Targeting alternative splicing to modulate nucleus REST: Potential implication for Huntington's disease

Authors: *G.-L. CHEN, Q. MA, D. GOSWAMI, H. YANG, G. MILLER;
Harvard Med. Sch/NEPRC, SOUTHBOROUGH, MA

Abstract: The repressor element silencing transcription factor (REST) acts as an orchestrator of the cellular epigenome and is implicated in many human diseases including Huntington's disease (HD) - a genetic neurodegenerative disease caused by expanded polyQ repeats in the huntingtin (Htt) protein. In HD, the mutant Htt loses association with cytoplasmic REST, resulting in enhanced REST nuclear transportation and increased nucleus REST which in turn represses neuronal gene expression involved in the survival and/or function of specific neurons. We have previously demonstrated that *REST* undergoes extensive alternative splicing, of which a common pattern of splicing - exon 3 (E3) skipping - causes loss of a domain essential for nuclear targeting and is linked to various types of cancer while it is pharmacologically manipulable. This study aims to examine whether E3 skipping can be targeted to reduce nucleus REST and rescue REST-mediated neuronal gene repression in a cellular model of HD. The striatal-derived mouse $STHdh^{Q7/Q7}$ and $STHdh^{Q111/Q111}$ cells, which express wild-type and mutant Htt, respectively, were treated with antisense oligos (ASOs) that mask splicing sites of *REST* E3. We found that: 1) ASOs treatment significantly induced E3 skipping and reduced nucleus REST, while it altered transcription and/or pre-mRNA splicing of specific neuronal genes in $STHdh^{Q111/Q111}$ cells; and 2) gene regulation effects of the ASOs were mimicked by siRNA-mediated knock-down of REST expression. The ASOs can also induce *REST* E3 skipping in mouse primary neurons and cell lines derived from rat and primates. Accordingly, our findings strongly suggest that

alternative *REST* splicing (especially E3 skipping) represents a novel, promising therapeutic target for HD and other diseases related to REST dysfunction.

Disclosures: **G. Chen:** None. **Q. Ma:** None. **D. Goswami:** None. **H. Yang:** None. **G. Miller:** None.

Nanosymposium

115. Spinal Cord Injury: Repair and Rehabilitation

Location: 152B

Time: Sunday, November 16, 2014, 8:00 AM - 9:45 AM

Presentation Number: 115.01

Topic: D.10. Spinal Cord Injury and Plasticity

Title: Progressive cervical spinal cord compression injury leads to compensatory neuroplasticity of the spinal respiratory circuitry

Authors: ***K. SATKUNENDRARAJAH**[1], S. K. KARADIMAS[2], M. G. FEHLINGS[1]; [1]Genet. and Develop., Toronto Western Res. Inst., Toronto, ON, Canada; [2]Inst. of medical science, Univ. of Toronto, Toronto, ON, Canada

Abstract: Spinal cord injury induced disruption of supraspinal and cervical respiratory circuitry has been shown to elicit compensatory functional and anatomical plasticity of the respiratory neural network. Most of this respiratory plasticity has been demonstrated in an acute left C2 hemisection injury (C2Hx) model that disrupts bublospinal inputs and silences ipsilateral respiratory motor output from phrenic motoneurons (PMN) located in the cervical spinal cord (C3-C6). Following this injury there is a partial restoration of ventilation over weeks that has been associated with morphological functional alterations suggesting a role for endogenous mechanisms of compensatory respiratory neuroplasticity. We hypothesized that, chronic progressive compression of the cervical spinal cord that results from cervical spondylotic myelopathy (CSM), elicits a distinct form of plasticity of the respiratory circuitry that prevents significant decline of respiratory function in this common clinical condition. To test this hypothesis, we utilized a novel clinically relevant model of CSM, where the cervical spinal cord is compressed progressively by the insertion of a biomaterial underneath the C4-C5 laminae above the PMNs. A sham group of mice underwent the same surgery without material implantation and chronic compression. In order to neurophysiological assess chronic compression induced respiratory plasticity, we performed a C2Hx injury at 2, 4, and 8 weeks post-material implantation and evaluated respiratory related diaphragmatic EMG activity. In sham-operated mice, C2Hx immediately silenced the ipsilateral phrenic motor output. While,

mice that had chronic compression of the spinal cord did not display a complete loss of ipsilateral phrenic motor output following C2Hx. Interestingly, the extent of respiratory related ipsilateral diaphragmatic activity maintained following C2Hx was progressively augmented with increase compression duration. In addition, we explored the neuroanatomical substrates that mediate this unique form of neurophysiological plasticity through detail analysis of morphological and functional alterations in spinal respiratory circuitry. In conclusion, these results reflect chronic compression induced dynamic changes in respiratory circuitry that manifests as compensatory respiratory behavior.

Disclosures: K. Satkunendrarajah: None. **S.K. Karadimas:** None. **M.G. Fehlings:** None.

Nanosymposium

115. Spinal Cord Injury: Repair and Rehabilitation

Location: 152B

Time: Sunday, November 16, 2014, 8:00 AM - 9:45 AM

Presentation Number: 115.02

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Wings for Life, Spinal Cord Foundation (WFL-US-004/11)

Title: Reticulospinal plasticity promotes skilled hand function recovery in rats with spinal cord injury

Authors: *G. GARCIA-ALIAS, K. TRUONG, P. SHAH, H. ZHONG, R. R. ROY, V. R. EDGERTON;
Integrative Biol. & Physiol., UCLA, Los Angeles, CA

Abstract: The corticospinal and rubrospinal tracts predominantly control skilled hand function. Injuries to these tracts impair grasping but not gross motor functions such as overground locomotion. The aim of the present study was to determine whether or not control via the reticulospinal tract could mediate skilled hand function after damage to both the corticospinal and rubrospinal tracts. Adult rats received a bilateral injury to the corticospinal tract at the level of the medullar pyramids and a bilateral ablation of the rubrospinal axons at C4. One group of rats received two injections of chondroitinase-ABC at C7 acutely after injury and then were trained daily for reaching and grasping rehabilitation beginning 7 days post-injury for 6 weeks (Chase group, n = 5). A second group of rats received analogous injections of ubiquitous penicillinase but did not undergo any training (Pen group, n = 5). Compared to rats in the Pen group, rats in the Chase group improved their reaching and grasping abilities over time and had

an increased density of reticulospinal processes in both the normal and ectopic areas of the grey ventral matter of the caudal segments cervical spinal cord. Overground locomotion was mildly and similarly affected in both groups. The results indicate that after damage to spinal tracts controlling specific functions, other related spinal tracts can take over the role of those damaged tracts and promote task specific recovery.

Disclosures: G. Garcia-Alias: None. **K. Truong:** None. **P. Shah:** None. **H. Zhong:** None. **R.R. Roy:** None. **V.R. Edgerton:** None.

Nanosymposium

115. Spinal Cord Injury: Repair and Rehabilitation

Location: 152B

Time: Sunday, November 16, 2014, 8:00 AM - 9:45 AM

Presentation Number: 115.03

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Shaw Foundation

Title: Pediatric constraint induced movement therapy for brachial plexus injury: Short and long term effcts of a randomized study

Authors: *T. KARAKOSTAS[1,2], E. C. KING[3,2], S. HSIANG[4];
[1]Motion Analysis Ctr., Rehabil. Inst. of Chicago, Chicago, IL; [2]Orthopaedic Surgery, Feinberg Sch. of Med., Chicago, IL; [3]Orthopaedics, Lurie Childrens Hosp. of Chicago, Chicago, IL; [4]Industrial Engin., Texas Tech. Univ., Lubbock, TX

Abstract: In the past we reported upper and lower extremity function changes as a function of a pediatric constraint induced movement therapy (pCIMT) camp for children with brachial plexus injury (BPI). Our reporting focused on pre- and post- performance of the experimental and control group. The objectives of this study were to a) contrast the immediate and long term performance of the experimental and control group, b) determine potential corticoplasticity related effects, and c) determine retention of these effects. This is a randomized control study including 17 children with BPI, 3-7 years of age, 9 of them randomly assigned in the experimental group (EG). No participant had history of other neuromusculoskeletal injury or CIMT exposure. All subjects could use the affected arm as gross assist during play and self care tasks. Cognitively, they could follow two step commands. Treatment took place at a Children's Hospital. We delivered 30 hours of treatment (3 hours of treatment specific training over 10 days). Activities focused on gross, fine motor and self feeding skills. Control group (CG)

participants had traditional occupational therapy (OT). Outcomes were measured using the Shrinner Hospital Upper Extremity Evaluation (SHUEE) and the GAITRite to assess gait. Participants in EG were tested pre post and six months post pCIMT. Participants in CG were tested pre and post 30 hours of treatment (six months). Results were initially explored with discriminant analysis and then for simplicity and reporting with t-tests ($a<0.05$). Table 1 presents selected results based on the SHUEE and GAITRite that showed significant changes in either EG or CG. **Table 1. Means, standard deviations and p values for selected output parameters.**

Parameter	**Pre-EG**	**Post-EG**	**p**	**6Post-EG**	**p**	**Pre-CG**	**Post-CG**	**p**
	Mean(SD)	*Mean(SD)*		*Mean(SD)*		*Mean(SD)*	*Mean(SD)*	
Spontaneous Functional Analysis	*71.6(9.9)*	*86.5(10.3)*	.00	73.4*(13.8)*	.07	68.5*(12.6)*	73.4*(16.7)*	.36
Dynamic Positional Analysis	*62.9(12)*	79.7*(9.1)*	.00	76.2*(8.6)*	.25	69.6*(9.3)*	74.2*(11.1)*	.15
Velocity (normalized)	3.5*(.8)*	3.9*(.7)*	.02	4*(.6)*	.81	3.9*((0.2)*	4.1*(0.8)*	.6
Cadence (normalized)	293.5*(30)*	313.4*(27)*	.01	328.6*(30)*	.01	308.2*(19)*	323.2*(39)*	.36
Step length difference (cm) (involved vs. uninvolved)	**1.0*(.3)***	**0.5*(.4)***	**.02**	**0.6*(.6)***	**.59**	**0.9*(0.7)***	**1.9*(0.9)***	**.18**

This is, to our knowledge, the first randomized control study investigating the immediate and long term effects of pCIMT on the function of upper and lower extremities of children with BPI. The results demonstrate clear improvements and retention in the upper and lower extremity function. The results also suggest superiority of pCIMT over the traditional OT approach in treating functional deficits for children with BPI.

Disclosures: T. Karakostas: None. **E.C. King:** None. **S. Hsiang:** None.

Nanosymposium

115. Spinal Cord Injury: Repair and Rehabilitation

Location: 152B

Time: Sunday, November 16, 2014, 8:00 AM - 9:45 AM

Presentation Number: 115.04

Topic: D.10. Spinal Cord Injury and Plasticity

Title: The integration of vision and proprioception on obstacle crossing strategies in people with motor-incomplete spinal cord injury

Authors: R. N. MALIK, *T. LAM;
Univ. British Columbia, Vancouver, BC, Canada

Abstract: Background: In people with motor-incomplete spinal cord injury (m-iSCI), the ability to perform skilled walking tasks (such as obstacle crossing) is an essential component of functional mobility. Sensorimotor integration of visual and proprioceptive inputs is important for successful obstacle crossing. In people with m-iSCI, proprioceptive information could be compromised, but we know very little about the impact of proprioceptive deficits on the performance of obstacle crossing. Thus, the overall objective of this study is to understand how motor and sensory deficits in people with m-iSCI affect obstacle-crossing strategies. **Methods:** Individuals with m-iSCI and able-bodied controls performed an obstacle-crossing task. Participants walked along a walkway between parallel bars and stepped over an obstacle with either full or obstructed vision. An eye tracker was used to determine gaze behavior (gaze duration and number of glances to the obstacle), and motion capture analysis was used to determine lead and trail limb horizontal distance from the obstacle and lead toe clearance height over the obstacle. In subjects with SCI, manual muscle strength, the Spinal Cord Injury-Functional Ambulation Profile (SCI-FAP), and the 10-meter walk test was used to assess motor capacity. Lower limb proprioceptive sense was assessed using a hip and knee joint position-matching task using the Lokomat and customized software controls. **Results:** Lower limb proprioceptive sense was varied across subjects with m-iSCI. In general, m-iSCI participants tended to glance at the obstacle more frequently with longer gaze durations compared to controls. When the lower visual field was obstructed, able-bodied controls exhibited increasing lead and trail limb horizontal distance and toe-obstacle clearance (consistent with previous studies). In subjects with m-iSCI, lead and trail limb horizontal distance and toe-obstacle clearance height tended to be smaller and was modulated to a lesser extent with visual field occlusion compared to that measured in controls. Subjects with m-iSCI also showed more trial-to-trial variability in these gait parameters compared to controls. **Conclusion:** The results of this study indicate that people with SCI rely more heavily on vision to cross obstacles and show limited ability to modulate the key gait parameters required for successful obstacle crossing. Our data suggest that proprioceptive deficits also need to be considered in rehabilitation programs aimed at improving functional mobility in individuals with m-iSCI.

Disclosures: R.N. Malik: None. **T. Lam:** None.

Nanosymposium

115. Spinal Cord Injury: Repair and Rehabilitation

Location: 152B

Time: Sunday, November 16, 2014, 8:00 AM - 9:45 AM

Presentation Number: 115.05

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Grant 062009

NIH Grant EB007615-03

Russian Grant 01172

Russian Grat 12074-OFI-M-2011

Title: Mechanisms underlying the interactive effects of multi-site epidural spinal cord stimulation in spinal rats

Authors: *P. SHAH[1], S. SUREDDI[1], M. ALAM[1], P. GAD[1], H. ZHONG[1], R. ROY[1], V. R. EDGERTON[1], Y. GERASIMENKO[2];
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Abstract: Epidural spinal cord stimulation (ES) at spinal cord segments L2 or S1 either in a monopolar or bipolar combination facilitates stepping in spinal rats via neuromodulation of the locomotor networks in the lumbosacral region of the spinal cord. The mechanisms underlying the interaction of multi-site stimulation to facilitate stepping, however, remain unknown. We have demonstrated that hindlimb stepping in spinal rats is more robust with simultaneous and independent monopolar epidural stimulation (SIM-ES) at L2 (40 Hz) and S1 (10, 20, and 40 Hz) spinal segments with monopolar or bipolar ES. Additionally, there is greater activation of the extensor muscles (medial gastrocnemius) with SIM-ES vs. bipolar ES. Collectively, these data suggest that postural control during locomotion is partly exerted via neuronal networks located in the sacral region of the spinal cord. We now elucidate the electrophysiological mechanisms underlying the interactive effects of independent stimulation pulses at L2 and S1 during stepping in spinal rats. Several hindlimb muscles of ten adult female rats were implanted bilaterally with chronic intramuscular recording electrodes. After a mid-thoracic spinal cord transection (ST) the rats underwent bipedal step training on a treadmill for 30 min/day, 3 days/wk for 8 sessions in the presence of bipolar L2-S1 or SI-L2 stimulation. Twenty-five days post-ST, EMG activity and bipedal stepping ability were assessed using nine combinations of ES at L2 (40 Hz) and S1 (5, 10, 15, 20, and 40 Hz). Our results indicate that with a progressive increase in stimulation frequency at the sacral segment from 5 to 40 Hz while keeping the stimulation frequency at L2

constant (40 Hz) during stepping: a) the cumulative integral of individual evoked responses from S1 increased, whereas the evoked responses from L2 remained the same, b) the evoked responses from L2 were phase specific, e.g., appearing, only during the stance phase in the medial gastrocnemius, whereas the responses from S1 were modulated throughout the step cycle. We also found that the inter-pulse interval between the two stimulation pulses might be one of the determining factors of step quality. Our data suggest that the neuronal networks in the L2 spinal segment serve as "controllers" for spinal bipedal stepping, whereas the S1 neuronal pool is likely a "modulator" of spinal stepping. Our findings from this simple SIM-ES paradigm of stimulation at only two sites along the spinal cord have a direct impact in the development and use of complex spinal ES array systems both in the rat and human.

Disclosures: P. Shah: None. **S. Sureddi:** None. **M. Alam:** None. **H. Zhong:** None. **R. Roy:** None. **V.R. Edgerton:** None. **Y. Gerasimenko:** None. **P. Gad:** None.

Nanosymposium

115. Spinal Cord Injury: Repair and Rehabilitation

Location: 152B

Time: Sunday, November 16, 2014, 8:00 AM - 9:45 AM

Presentation Number: 115.06

Topic: D.10. Spinal Cord Injury and Plasticity

Title: Stimulation-induced pendulate tail: Evidence exposure to fixed spaced shock engages a spinal oscillator

Authors: *M. M. STRAIN, B. S. PESEK, Y.-J. HUANG, C. R. STUMP, J. D. TURTLE, K. H. LEE, J. W. GRAU;
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Abstract: The temporal distribution of stimulation has been shown to impact spinal cord plasticity after spinal cord injury. Specifically, stimuli presented in a regular (fixed spaced) or irregular (variable) spaced manner have divergent effects on spinal cord plasticity. Just six minutes of variable spaced stimulation produces a maladaptive effect that inhibits adaptive plasticity. In contrast, fixed spaced stimulation promotes adaptive plasticity and can reverse and prevent the effects of variable spaced stimulation. The effects of fixed space stimulation require extended training and depend on NMDA and BDNF signaling. Recent studies in our lab provide evidence that the beneficial effects of fixed spaced shock also depend upon the central pattern generator within the lumbar enlargement. Given the role of the central pattern generator in locomotor behavior, we next examined whether the temporal distribution of shock has

differential effects on locomotion. To examine the effects of shock on locomotor performance, spinally transected rats were given an intrathecal injection of a cocktail of serotonin (5HT) and NMDA to induce air stepping. Two minutes after injection, subjects received 720 80-msec shocks in either a variable (0.2-3.8 s) or fixed (every 2 s; 0.5 Hz) spaced manner. Other groups remained unshocked or received a continuous shock. The hindlimbs of subjects were recorded both during the shock period and for 30 minutes after shock had ended. Bilateral stepping behavior was scored using video playback. We observed sustained stepping in subjects that received fixed spaced shock over relative to groups that received variable spaced or no shock. Unexpectedly, in some subjects that received fixed spaced shock, the tail began to move in an oscillating fashion (Pendulate Tail). Tail swinging continued after shock exposure and had the same frequency as fixed spaced shock (0.5 Hz). Thus, fixed spaced shock treatment seems capable of entraining tail oscillation when given in combination with 5HT and NMDA. The oscillation of the entrained pendulate tail lasted more then an hour after the conclusion of the shock. Further, fixed-spaced shock administered to either the leg or the tail entrained tail oscillations. Additionally, immobilization of the tail had little effect on the subsequent oscillations of the tail and upon release, the tail resumed oscillation in phase with oscillations prior to immobilization. These observations provide strong evidence for a spinal oscillator that can be entrained by external stimulation.

Disclosures: **M.M. Strain:** None. **B.S. Pesek:** None. **Y. Huang:** None. **C.R. Stump:** None. **J.D. Turtle:** None. **K.H. Lee:** None. **J.W. Grau:** None.

Nanosymposium

115. Spinal Cord Injury: Repair and Rehabilitation

Location: 152B

Time: Sunday, November 16, 2014, 8:00 AM - 9:45 AM

Presentation Number: 115.07

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH 1F31NS084723

NIH R01 NS079751

Foundation for Physical Therapy - PODS II Scholarship

Chicago Biomedical Consortium - Scholar Award

Title: Immediate and sustained effects of high-intensity locomotor practice on gait performance in individuals with incomplete spinal cord injury

Authors: *K. A. LEECH[1], C. L. HOLLERAN[2], C. R. KINNAIRD[2], T. G. HORNBY[3,2]; [1]SMPP, Northwestern Univ. / Rehabil. Inst. of Chicago, Chicago, IL; [2]Sensory Motor Performance Program, Rehabil. Inst. of Chicago, Chicago, IL; [3]Univ. of Illinois at Chicago, Chicago, IL

Abstract: Recent evidence suggests that intensity of practice may be a critical component to improve locomotor function following neurologic injury. To date, high intensity locomotor exercise has largely been avoided in rehabilitation following neurologic injury, possibly due to the traditional theory that such activities will lead to aberrant movement patterns and spastic motor behaviors. However, the immediate effect of locomotor exercise intensity on gait performance has not been rigorously evaluated and no studies demonstrate a negative impact following high-intensity locomotor training. The aim of this study was to evaluate the immediate effects of exercise intensity on key measures of gait performance, with an emphasis on muscle timing, gait variability (SD and COV between step cycles), and consistency of intralimb coordination in subjects with motor incomplete SCI. A secondary aim was to determine the impact of high intensity training on these measures. We evaluated the effect of varied levels of intensity on locomotor performance in 19 subjects with chronic incomplete SCI. Subjects performed a graded-intensity locomotor exercise task with simultaneous collection of lower extremity EMG and kinematics. Following initial testing, a subset of 10 subjects participated in 12 weeks of high intensity locomotor training, with similar measures reassessed post-training. To evaluate the short-term intensity-dependent changes in gait performance, comparisons were made across levels of intensity (low=33%, moderate=66%, and high=100% of peak gait speed) with a repeated measure ANOVA. Training effects were assessed by a comparison between measures at the highest speed common to pre- and post-training testing using a paired T-test. Our results indicate no change in muscle timing or gait variability across levels of intensity. Intralimb coordination was found to be significantly more consistent at moderate versus low intensity ($p<0.01$); this effect tended to be maintained at high intensity ($p=0.06$). In addition, high intensity training led to a significant improvement in overall locomotor performance (increased peak gait speed from 0.61 to 0.74 m/s; $p<0.01$) with no change in coordination and a trend for decreased variability in ankle range of motion ($p=0.07$). In contrast to the theoretical framework of traditional rehabilitation, our data demonstrate that high intensity locomotor exercise does not elicit significantly variable movement patterns or aberrant muscle activity during walking. Furthermore, repeated exposure to high-intensity locomotor practice may improve the consistency of gait kinematics and coordination.

Disclosures: K.A. Leech: None. **C.L. Holleran:** None. **C.R. Kinnaird:** None. **T.G. Hornby:** None.

Nanosymposium

116. Consequences and Mechanisms of Exposure to Stressors

Location: 147B

Time: Sunday, November 16, 2014, 8:00 AM - 10:00 AM

Presentation Number: 116.01

Topic: E.05. Stress and the Brain

Support: NIH Grant R01MH087495

Brains Grant

CIRM fellowship

Title: Adverse early life environment suppresses neurogenesis and induces oligodendrogenesis in the hippocampus

Authors: *K. TARAVOSH-LAHN[1], S. Y. KIM[1], K. LONG[1], D. MILIKOVSKY[3], D. FRANCIS[2], D. KAUFER[1];
[1]Integrative Biol., [2]Publ. Hlth., Univ. of California, Berkeley, Berkeley, CA; [3]Dept. of Physiol. & Cell Biology,Cognitive & Brain Sciences,Zlotowski Ctr. for Neurosci., Ben-Gurion Univ. of the Negev, Beer-Sheva, Israel

Abstract: Adult hippocampal neurogenesis is suppressed by exposure to stress and the stress hormone glucocorticoids (GCs). In vitro, treatment of neural precursor cells (NPCs, the multipotent stem cells of the hippocampal neurogenic niche) with GCs induces a pro-oligodendrogenic transcriptional program and results in a shift in differentiation from a neuronal to oligodendrocytic fate. In the adult dentate gyrus, one week of restraint stress or GC exposure induced a similar increase in pro-oligodendrogenic transcription program and increase in newborn oligodendrocytes. We examined the effects of early life stress on oligodendrogenesis and associated changes in white matter. Perinatal exposure to stressful environment or GCs lead to similar increases in white matter and myelin-related genes. Differential maternal care resulted in changes in white matter in several brain regions including the hippocampus and corpus callosum. Together, these results suggest a novel model in which stress may alter brain function by promoting oligodendrogenesis, thereby altering the cellular composition and white matter structure of the brain.

Disclosures: **K. Taravosh-Lahn:** None. **S.Y. Kim:** None. **K. Long:** None. **D. Francis:** None. **D. Kaufer:** None. **D. Milikovsky:** None.

Nanosymposium

116. Consequences and Mechanisms of Exposure to Stressors

Location: 147B

Time: Sunday, November 16, 2014, 8:00 AM - 10:00 AM

Presentation Number: 116.02

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH-MH60670

University of Michigan Department of Anesthesiology

Title: Exaggerated changes in REM and transition to REM sleep states in response to fear conditioning following traumatic stress exposure

Authors: *W. M. VANDERHEYDEN, L. M. URPA, G. R. POE;
Univ. of Michigan, Ann Arbor, MI

Abstract: Post-traumatic stress disorder (PTSD) is accompanied by sleep disturbances and fear-associated memory impairments. REM sleep has been linked with emotional processing while REM sleep and the sleep spindle-rich transition to REM sleep states are both associated with memory consolidation. We hypothesized that a rodent model of PTSD would exhibit impaired sleep responses in a fear conditioning and extinction task and that these altered responses would predict the negative cognitive consequences. 18 adult male Sprague Dawley rats were surgically implanted with skull EEG and nuchal EMG electrodes for continuous recording. and state assessment. Ten animals were exposed to Single Prolonged Stress (SPS) trauma then continuously recorded for 8 days before being exposed to fear conditioning. Eight yoked control animals were recorded simultaneously without exposure to SPS trauma under the same fear conditioning paradigm. Fear conditioning consisted of 5 exposures to 1 mAmp shock paired with an 80 dB tone in Context A.The following day extinction training consisted of 30 of the same tone exposures in Context B. Finally, on the third day, recall training consisted of 10 re-exposures to Context B. EEG and EMG recordings were analyzed for 24 h following tone presentations each day. Statistical comparisons of sleep states and sleep architecture were made between SPS and control animals that were fear conditioned. The normal response to fear conditioning in control animals was REM suppression ($p < 0.05$) compared with their own baseline. However, SPS treated rats showed individual differences in susceptibility to extinction recall deficits. Similar to controls, resistant animals (freezing 50% of total time on extinction recall day) ($p<0.05$). Transition to REM (TR) sleep more than doubled from post-trauma baseline in response to fear conditioning in all SPS treated animals ($p<0.01$), however susceptible rats obtained less TR sleep than resistant rats after fear conditioning ($p=0.02$) and

extinction (p=0.03) treatments. No significant differences in waking or non-REM sleep distinguished SPS from control groups. Thus, in the manner of reversal learning deficits (Watts et al, J Neurosci 2012), fear extinction consolidation deficits correlated with both REM sleep responses and TR sleep levels. Future experiments will explore mechanisms at work during both TR and REM sleep, such as LC silence, spindle, theta and gamma rhythms, that likely allow depotentiation and rewiring in memory circuits involved in contextual discrimination learning in the extinction of fear.

Disclosures: W.M. Vanderheyden: None. **G.R. Poe:** None. **L.M. Urpa:** None.

Nanosymposium

116. Consequences and Mechanisms of Exposure to Stressors

Location: 147B

Time: Sunday, November 16, 2014, 8:00 AM - 10:00 AM

Presentation Number: 116.03

Topic: E.08. Biological Rhythms and Sleep

Support: NIH R01 MH063266

Title: Effects of chronic social defeat stress on sleep, body temperature, and motor activity in mice

Authors: *A. M. WELLS, H. PANTAZOPOULOS, R. J. DONAHUE, C. J. WEBBER, W. A. CARLEZON, Jr.;
Psychiatry, Harvard Med. School, McLean Hosp., Belmont, MA

Abstract: Stress is a major contributing factor to the development and persistence of psychiatric illnesses like Major Depressive Disorder (MDD) and Post-Traumatic Stress Disorder (PTSD). Understanding how stress produces hallmark symptoms of mood disorders will facilitate the development of treatments designed to reduce the severity and duration of these illnesses. Two core symptoms that manifest in MDD, PTSD, and other neuropsychiatric disorders are altered sleep architecture and circadian rhythm (processes that follow an entrainable, 24-h oscillation). In rodents, restraint stress and exposure to an aggressive conspecific (social defeat) similarly increase non-rapid eye movement (NREM) sleep duration and intensity, but differentially affect rapid eye movement (REM) sleep. However, it is currently unknown if chronic, repeated stress in mice produces comparable or unique adaptations in sleep. The present study was designed to evaluate the effects of chronic social defeat stress (CSDS), a behavioral paradigm that engenders a long-lasting depressive-like phenotype in mice, on sleep, body temperature, and activity. Adult

male C57BL6/J mice were surgically implanted with telemetry transmitters that permit continuous wireless recording of cortical EEG, neck EMG, body temperature, and activity. After recovery from surgery, baseline recordings were obtained for 5 days, followed by exposure to a 10-day CSDS (or control) regimen. On each day, 1 h into the light cycle (zeitgeber time 1), defeated mice were exposed to a novel, aggressive CD-1 mouse for 10 min, followed by continuous, protected sensory exposure. Control mice were also housed opposite a conspecific with continuous, protected sensory exposure, but physical contact never occurred. Analysis of time spent in NREM sleep per 24-h cycle (percentage of pre-defeat baseline) across the 10 days of CSDS revealed the emergence of a dichotomous population in defeated mice (i.e., sensitive and insensitive). Sensitive mice exhibited increases in NREM time, relative to controls, independent of the number of defeat sessions experienced. In contrast, there was no difference in NREM time between insensitive and control mice. Moreover, there were no differences in REM time or in NREM or REM bouts among the groups. Ongoing work will 1) quantify CSDS-induced changes in body temperature and activity, 2) correlate changes in these metrics and in NREM sleep with other behavioral endpoints of CSDS (e.g., social avoidance) to characterize the relationship between circadian modifications and the severity of depressive-like phenotypes, and 3) explore the neural mechanisms by which CSDS alters NREM sleep.

Disclosures: A.M. Wells: None. **H. Pantazopoulos:** None. **R.J. Donahue:** None. **C.J. Webber:** None. **W.A. Carlezon:** None.

Nanosymposium

116. Consequences and Mechanisms of Exposure to Stressors

Location: 147B

Time: Sunday, November 16, 2014, 8:00 AM - 10:00 AM

Presentation Number: 116.04

Topic: E.05. Stress and the Brain

Title: Role of DNA methylation in the synaptic and behavioral effects of long-term severe stress

Authors: *J. CHENG, J. WEI, Z. YAN;
UB, Buffalo, NY

Abstract: Chronic severe stress acts as a trigger for multiple mental disorders, however, the mechanisms underlying the stress-induced sustained changes remain elusive. In this study, we found that adolescent male rats subjected to repeated severe stress (restraint 6 hrs/day for 7 days) exhibited impaired recognition memory, elevated aggression and anxiety-like behaviors. Moreover, they had significantly suppressed AMPAR-mediated excitatory postsynaptic currents

(AMPAR-EPSC) and AMPAR subunit expression in the prefrontal cortex (PFC). Many of these behavioral, physiological, and biochemical changes remained even 7 days after the termination of stress. A decreased mRNA level of DNA methylthansferase 3a (DNMT3a) was observed in the PFC of stressed animals, which seemed to be responsible for the reduced GluR2 expression and AMPAR-EPSC. Our data suggest that long-term severe stress may induce sustained changes in synaptic transmission via DNA methylation, which may contribute to the behavioral abnormality related to mental disorders.

Disclosures: J. Cheng: None. **J. Wei:** None. **Z. Yan:** None.

Nanosymposium

116. Consequences and Mechanisms of Exposure to Stressors

Location: 147B

Time: Sunday, November 16, 2014, 8:00 AM - 10:00 AM

Presentation Number: 116.05

Topic: E.05. Stress and the Brain

Support: NIH Grant MH85774

Title: The epigenetic mechanism of repeated stress in prefrontal cortex

Authors: *J. WEI, Z. XIONG, J. B. LEE, J. CHENG, L. J. DUFFNEY, E. R. MATAS, Z. YAN;
Physiol. & Biophysics, State Univ. of New York at Buffalo, Buffalo, NY

Abstract: Stress and the major stress hormone, corticosterone, induce profound influences in the brain. Altered histone modification and transcriptional dysfunction have been implicated in stress-related mental disorders. Our previous studies have found that repeated stress impairs prefrontal cortex (PFC)-mediated cognitive functions by increasing the ubiquitination and degradation of AMPA-type glutamate receptors via a mechanism depending on the E3 ubiquitin ligase Nedd4. Here we found that in PFC of repeatedly stressed animals, active GR had increased binding to the glucocorticoid response element (GRE) of Histone Deacetylase 2 (HDAC2) promoter, resulting in the upregulation of HDAC2. Consequently, the histone methyltransferase G9a is suppressed, leading to the loss of repressive histone methylation at Nedd4 promoter and increased transcription of Nedd4. Inhibition or knockdown of HDAC2 blocked the stress-induced impairment of PFC synaptic transmission, AMPAR expression and recognition memory. These results have provided an epigenetic mechanism and a potential rescue strategy for the detrimental effects of chronic stress.

Disclosures: **J. Wei:** None. **Z. Xiong:** None. **J.B. Lee:** None. **J. Cheng:** None. **L.J. Duffney:** None. **E.R. Matas:** None. **Z. Yan:** None.

Nanosymposium

116. Consequences and Mechanisms of Exposure to Stressors

Location: 147B

Time: Sunday, November 16, 2014, 8:00 AM - 10:00 AM

Presentation Number: 116.06

Topic: F.02. Animal Cognition and Behavior

Support: HHMI

Base grant P51RR000165 to Yerkes National Primate Research Center

Title: microRNA and consolidation of cued fear memory

Authors: ***B. G. DIAS**, J. GOODMAN, R. AHLUWALIA, A. E. EASTON, R. A. GALI, K. RESSLER;
Emory Univ., Atlanta, GA

Abstract: Consolidation of memory requires gene regulation and protein synthesis. In recent times, short non-coding RNAs have garnered significant attention in their ability to affect the aforementioned molecular processes. We chose to address how a specific species of these RNAs, microRNA (miRNA), might impact the consolidation of cued fear memory in an auditory fear conditioning task. miRNA microarray and independent expression analysis revealed miR-34a to be significantly increased in amygdala tissue obtained 30 mins after auditory fear conditioning of adult mice. Bioinformatics analyses suggested components of the Notch pathway to be predicted targets of these miRNA. Using luciferase-based cell culture techniques, we validated Notch1 as a bonafide target of miR-34a. Querying expression levels of Notch pathway components in the amygdala indicated a decrease in mRNA and protein levels after auditory fear conditioning. We then carried out gain- and loss-of-function studies by manipulating miRNA action and target gene function to understand the relationship between miRNA and target gene function in the consolidation of cued fear memory. Inhibiting miR-34a function in the BLA impaired the consolidation of fear memory, while inhibiting Notch function in the BLA enhanced this consolidation. Our data indicate that miR-34a decreases Notch signaling in the amygdala after fear conditioning thereby creating a molecular genetic microenvironment that facilitates the consolidation of fear memory. This work adds to accumulating literature which suggests that developmental molecules serve important functions in the adult nervous system. Finally, with the

Notch pathway being a target of cancer therapeutics, perhaps co-opting existing pharmacological strategies to modulate Notch signaling might be a viable treatment avenue for the enhanced consolidation of fear memories that are often debilitating to quality of life.

Disclosures: B.G. Dias: None. **J. Goodman:** None. **R. Ahluwalia:** None. **A.E. Easton:** None. **R.A. Gali:** None. **K. Ressler:** None.

Nanosymposium

116. Consequences and Mechanisms of Exposure to Stressors

Location: 147B

Time: Sunday, November 16, 2014, 8:00 AM - 10:00 AM

Presentation Number: 116.07

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: MH096093

Beckman Institute

Title: Unbiased comprehensive analysis of neural activity in response to fear with *in vivo* MR imaging of animal models of PTSD

Authors: *A. GONZALES[1], A. DELORA[1], R. E. JACOBS[2], E. L. BEARER[1,2];
[1]Pathology, The Univ. of New Mexico, Albuquerque, NM; [2]Caltech, Pasadena, CA

Abstract: Post-traumatic stress disorder (PTSD) is a mental illness affecting 7.8% of the population. Many areas in the brain, including the amygdala, hippocampus, and the prefrontal cortex (PFC) have been implicated by fMRI in PTSD, and monoamine transporters are among the known targets for current interventions. Our group has assessed functional circuitry from PFC *in vivo* by tract tracing with manganese-enhanced magnetic resonance imaging (MEMRI) and correlation histology. These techniques identified alterations in the mesolimbic cortical circuitry of transgenic mice lacking the monoamine transporter genes, SERT, DAT and NET (Bearer et al. 2009, Zhang, Bearer et al. 2010, Gallagher et al. 2013). Here we report a new method that uses systemic manganese to image neural activity in response to fear, a validated approach to elicit PTSD-like symptomatology in rodents. Fear was provoked by exposure to predator odor (2,3,5-trimethyl-3-thiazoline (TMT), an odorant derived from the fox anal gland. Following intra-peritoneal injection of Mn^{2+}, mice (n=12) were first exposed to a control substance (saline) and then to TMT. Behavior was recorded with Ethovision in a light-dark box and statistically analyzed in R. An ANOVA of mouse behavior in the presence of each type of

odor demonstrated that TMT significantly induced more anxiety-like behavior, such as less time in the lighted part of the box, than saline ($p<0.005$). Whole brain, T_1-weighted images were acquired longitudinally: prior to Mn^{2+} injection, after Mn^{2+} injection, and at four consecutive time points over two hours post exposure to predator odor. A one-way within-subjects ANOVA comparing the MR scans immediately before and after the fear provocation detected increased Mn^{2+}-enhanced signal in the amygdala, hypothalamus, median raphe, and lateral septal nuclei ($n=12$, $p < 0.05$, FDR corrected) with evidence implicating these areas in PTSD, both in humans and rodents. Histology of same brains stained for c-Fos, a marker of neuronal activity, confirmed the MR detection. Thus, our new MR method reliably method reveals the murine fear-response pathway. This new methodology will allow us now to witness the evolution of neuronal activity in living mice following stressful experience. Supported by MH096093 (ELB) and the Beckman Institute (REJ).

Disclosures: **A. Gonzales:** None. **A. Delora:** None. **R.E. Jacobs:** None. **E.L. Bearer:** None.

Nanosymposium

116. Consequences and Mechanisms of Exposure to Stressors

Location: 147B

Time: Sunday, November 16, 2014, 8:00 AM - 10:00 AM

Presentation Number: 116.08

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant 5K12GM000680-14

Base Grant No. P51RR-000165

Title: 3,4-methylenedioxy-N-methylamphetamine (MDMA, ‘ecstasy’) enhances the extinction of cued fear memory in mice

Authors: *M. YOUNG, L. L. HOWELL;
Yerkes Natl. Primate Res. Ctr., Atlanta, GA

Abstract: While many studies in animals have investigated the detrimental effects of long-term and binge use of 3,4-methylenedioxy-N-methylamphetamine (MDMA, ‘ecstasy’) on cognition and brain function, recent clinical studies have reported that acute MDMA treatment can have long-term positive effects on symptoms of post-traumatic stress disorder (PTSD) when combined with psychotherapy. Understanding the mechanisms of MDMA's therapeutic effect is difficult because of the relative dearth of research on the neurobiological mechanisms through which

acute MDMA affects cognition. To begin to explore the mechanisms through which MDMA might facilitate psychotherapeutic treatment of PTSD, we used Pavlovian fear conditioning and extinction in mice as models of PTSD and subsequent exposure therapy. Animals were conditioned with either single or multiple tone-shock pairings. In both conditioning paradigms, administration of MDMA before sub-optimal extinction training enhanced the retention of extinction memory for up to at least 2 weeks. In addition, unlike saline-treated mice, MDMA-treated mice maintained cued fear memory extinction outside of the extinction context, suggesting that MDMA supports generalization of extinction learning. Using site-directed infusion, we demonstrate that the extinction-enhancing effect of MDMA appears to be mediated in part within the infralimbic cortex, a cortical structure previously observed to be important for extinction. MDMA has widespread effects on monoaminergic signaling in the central nervous system. To begin to identify which of MDMA's neuromodulatory effects facilitate extinction, we co-administered MDMA with a variety of pharmacological challenges before extinction training, each targeting a different potential site of action. These include the serotonin transporter (SERT), the norepinephrine transporter (NET), and the serotonin 2A receptor (5-HT2a). Together, these results point to a potential model of how MDMA facilitates the efficacy of psychotherapeutic treatment of PTSD.

Disclosures: M. Young: None. **L.L. Howell:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); National Institute of Drug Abuse.

Nanosymposium

117. Functional Mechanisms of Attention

Location: 150A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 117.01

Topic: F.01. Human Cognition and Behavior

Support: VIDI grant Netherlands Organization for Scientific Research

Title: Facilitation and inhibition in attention: Functional dissociation of pre-stimulus alpha activity, P1 and N1 components

Authors: *H. A. SLAGTER[1], S. PRINSSEN[1], L. C. RETEIG[1], M. X. COHEN[1], A. MAZAHERI[2];
[1]Dept. of Psychology, [2]Dept. of Psychiatry, Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: Attention - the ability to attend to some things while ignoring others - can be best described as an emergent property of many neural mechanisms, facilitatory and inhibitory, working together to resolve competition for limited processing resources and control of behavior. To gain a better understanding of how attentional inhibition and facilitation are neurally implemented, here, in two studies, participants continuously attended to one and the same hemifield during the entire experiment while their brain activity was recorded using EEG. In addition, stimuli were only ever presented at the attended location. We reasoned that the consistent assignment of relevance to one hemifield would allow us to better separate inhibitory and facilitatory effects of attention in the brain. Indeed, in striking contrast to previous studies which typically observed bilateral attentional modulations of early sensory processing when subjects alternated between attending left and right, in both studies, we found perfectly lateralized P1 and N1 attentional modulations to, respectively, ipsilateral (P1) and contralateral (N1) posterior scalp regions. These findings substantiate the idea that the P1 reflects inhibition and the N1 amplification. Moreover, in further contrast to previous studies, greater pre-stimulus alpha activity was observed over relevant vs. irrelevant posterior regions, supporting proposals that alpha power reflects active inhibition only required when irrelevant regions compete for attentional resources. Together, these findings suggest a functional dissociation between (top-down inhibition), the P1 (bottom-up inhibitory process), and the N1 (facilitation). They also highlight the influence of statistical task structure and expectations on attentional control dynamics.

Disclosures: H.A. Slagter: None. **S. Prinssen:** None. **L.C. Reteig:** None. **M.X. Cohen:** None. **A. Mazaheri:** None.

Nanosymposium

117. Functional Mechanisms of Attention

Location: 150A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 117.02

Topic: F.01. Human Cognition and Behavior

Support: NIH EY021644

NSF 1059523

Title: Viewing the real-world: The effect of semantic and syntactic object properties on visual attention

Authors: *G. L. MALCOLM, S. SHOMSTEIN;
Dept. of Psychology, The George Washington Univ., Washington, DC

Abstract: Humans preferentially attend to objects when viewing their environment, making object properties an integral component in constraining attentional allocation. Extensive research has investigated object properties in relation to space and low-level features, yet their influence in real-world settings has largely been ignored. This forms a critical gap in understanding the human attentional mechanism. Here we elucidate the effect of object semantics (their high-level meaning, e.g., car) and syntactic properties (their spatial arrangement) on attentional allocation. A series of experiments examined the influence of semantic properties by presenting an object at fixation and two in the periphery that varied in their relation to the fixated object (e.g., a mailbox at fixation and an envelope and light bulb in the periphery). Objects appeared for a duration ranging from 250-2000ms prior to target/distractors onsetting on the objects. Despite being task-irrelevant, semantic relatedness influenced attentional allocation, with semantically related objects facilitated early in the time course and inhibited later in the time course (consistent with inhibition-of-return). Additionally, the stability of this semantic influence was tested by probing for the effect in the presence of a spatial bias. A semantic influence was again observed, though it was now delayed. Our results demonstrate that semantic information affects attentional allocation in favor of semantically-related objects, and it does so in a robust fashion even when a more predictive factor is present (i.e., spatial probability). Syntactic properties were then examined in a series of eye-tracking experiments. Participants viewed scenes for 3s before a cue appeared on a surface. Once the cue was fixated, a target then appeared on the same or different surface as the cue (syntactic manipulation) or floating (violation). If syntactic relations affect attentional allocation, then the resulting eye movements and RT should be influenced accordingly. In particular, object based attention suggests that targets on the same surface as the cue should be responded to faster. Indeed, participants had shorter saccade initiations and RTs for same surface targets, but there was no difference between the different and floating conditions. The results demonstrate that objects' syntactic relationships affect attentional allocation, with objects sharing the same surface facilitated for processing. Taken together, the results suggest that the visual system continually utilizes semantic and syntactic object information to bias attentional allocation when viewing naturalistic displays.

Disclosures: G.L. Malcolm: None. **S. Shomstein:** None.

Nanosymposium

117. Functional Mechanisms of Attention

Location: 150A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 117.03

Topic: F.01. Human Cognition and Behavior

Support: In-kind EEG equipment support from the National Research Council of Canada

Title: Eeg auditory oddball tone processing as a predictor of cognitive workload states: The attenuating effect of task boredom

Authors: *K. VANBENTHEM[1], S. CEBULSKI[1], C. M. HERDMAN[1], J. KEILLOR[2]; [1]Carleton Univ., Ottawa, ON, Canada; [2]Natl. Res. Council of Canada, Ottawa, ON, Canada

Abstract: Adaptive automation makes use of known workload signatures associated with high cognitive load states, such as the reduced processing of deviant tones in auditory oddball paradigms. While it is intuitive to consider high cognitive load effects, low loads can also result in performance decrements. The current study examined the "boredom" effect on behavioural data and the processing of oddball tones by comparing two groups of subjects who differed in their difficulty-ratings for a match-to-sample task. All subjects completed the match-to-sample task in an easy and difficult condition with simultaneous exposure to task-irrelevant standard and deviant tones (10:1 ratio). One group of subjects rated the easy block as increasing in difficulty over the 20-minute timeframe. Reports by these subjects indicated that boredom with the task lead to a reduction in attention. A second group showed consistent difficulty-ratings for the easy task over time. Behavioural analysis revealed that the "bored" group (N=8) demonstrated significantly longer response times, $t=2.32$, $p=.03$, in the easy, but not the difficult task condition when compared to the non-bored group (N=11). Reduced accuracy (ns) was also found for the bored group in the easy task condition. Frontal EEG channels showed that the N1 and P2 components exhibited the expected differential in deviant versus standard tone processing in the easy task condition for both groups. The later (400-500 ms post-stimulus) sustained processing was only observed in the non-bored group. Standard tones were processed similarly between groups in the easy condition. Reduced processing of the deviant tone at 200 ms was shown in the bored group, as compared to the non-bored subjects. Similarly, after independent component decomposition, a temporal-occipital-sourced cluster showed reduced processing of the deviant tone at 200 ms in the bored group as compared to the non-bored subjects. Results provide evidence that the deleterious consequences of boredom resulting from very low cognitive load states are also evident in both channel and component space. Adaptive automation efforts can exploit well-studied EEG phenomena, such as deviant tone processing, to identify potentially high-risk periods in low cognitive load states.

Disclosures: K. Vanbenthem: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); National Research Council of Canada. **S. Cebulski:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); National Research Council of Canada. **C.M. Herdman:** C. Other Research Support (receipt of

drugs, supplies, equipment or other in-kind support); National Research Council of Canada. **J. Keillor:** None.

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117. Functional Mechanisms of Attention

Location: 150A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 117.04

Topic: F.01. Human Cognition and Behavior

Support: Medical College of Wisconsin Research Affairs Committee 3304871

National Institute of Aging Training Grant T35 AG029793-07

Title: Age-related changes in frontal cortex during high task demands

Authors: *K. BROWNING, S. PATEL, C. HUMPHRIES, M. SABRI;
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Abstract: It is well established that healthy older adults perform worse than younger adults during dual-task conditions compared to single-task controls. However, the neural basis of this deficit is largely unknown. We investigated the relationship between dual-task performance, functional brain activation, and structural changes in healthy aging using an auditory attention paradigm. FMRI was acquired in 18 younger (age 18 to 37 years) and 18 older adults (age 55 to 68 years) with normal hearing thresholds and normal cognitive function documented by neuropsychological testing (MMSE, WASI, and RBANS). Participants heard blocks of eight words and pseudowords spoken by a male or female voice. In each block, participants were instructed to identify either: (1) the gender of the speaker (male or female), (2) lexicality (word or pseudoword), or (3) a conjunction of both (dual-task; e.g., male and word/female and pseudoword). The older adults had a larger percent increase in reaction time during the dual-task compared to the single task (i.e., dual-task cost) than younger adults. During the dual-task, older adults exhibited a pattern of 'over-activation' in the frontoparietal network, bilaterally. When behavioral performance was included in the analysis, greater cost in the older adults was associated with increased brain activation in medial superior frontal cortex and lateral orbitofrontal/inferior frontal gyrus, regions implicated previously in monitoring performance and primary task goals. In concordance, greater cost was also associated with reduced gray matter thickness in these regions. In younger adults, brain activation in supramarginal gyrus/inferior

parietal sulcus covaried with cost. Together these results support a model of age-related compensatory mechanisms in frontal attention networks during high task demands.

Disclosures: **K. Browning:** None. **S. Patel:** None. **C. Humphries:** None. **M. Sabri:** None.

Nanosymposium

117. Functional Mechanisms of Attention

Location: 150A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 117.05

Topic: F.01. Human Cognition and Behavior

Support: Biotechnology and Biological Sciences Research Council, BBSRC U.K. (BB/J009849/1)

Title: Attentional modulation of repetition suppression effects in human face- and voice-sensitive cortex

Authors: ***Y. KIKUCHI**[1], J. IP[1], J. C. MOSSOM[1], N. BARRACLOUGH[2], C. I. PETKOV[1], Q. C. VUONG[1];
[1]Inst. of Neurosci., Newcastle Univ. Med. Sch., Newcastle Upon Tyne, United Kingdom; [2]Dept. of Psychology, Univ. of York, York, United Kingdom

Abstract: An important property of the brain is that its neurons reduce their responses to the repetition of the same or similar environmental stimuli. There is considerable interest in understanding how repetition suppression is influenced by attention, such as when people focus their attention on properties of the repeating stimuli. However whether comparable repetition effects operate in different sensory modalities and whether attention modulates repetition effects in a similar way across the modalities was unclear. We asked how attention modulates repetition effects in the auditory and visual modalities, either by changing the gain of repetition effects (affecting the intercept of the stimulus repetition function) or by selectively sharpening repetition effects (affecting the slope of the repetition function). Nine volunteers participated in separate auditory and visual fMRI experiments in which they directed their attention to voice or face identity changes or to changes of the respective stimuli in their spatial location. By morphing between pairs of different face or voice identities, we aimed to modulate the strength of repetition effects which are stronger for repetition of more similar stimuli. The spatial difference was manipulated by systematically changing the screen position for face pairs or the virtual acoustic location for voice pairs. We also equated performance on the identity and spatial tasks

across the two modalities. For each volunteer we functionally localised face- and voice-sensitive regions of interest (ROI). For both face and voice ROIs there was a significant change to the slope of the stimulus repetition function when volunteers attended to identity differences but not to spatial differences (interaction between attention and identity differences, $F(2,16)=4.9$, $p=.02$, but no interaction between attention and spatial differences, $F<1.0$). Moreover, the attentional modulation seemed to be specific to face/voice-sensitive cortex because it was not evident in temporal lobe areas outside of these ROIs. Overall the results suggest comparable repetition effects and attentional modulations of these effects in human face- and voice-sensitive cortex. CIP and QCV: joint senior authors; JI and JCM contributed equally.

Disclosures: Y. Kikuchi: None. **J. Ip:** None. **J.C. Mossom:** None. **N. Barraclough:** None. **C.I. Petkov:** None. **Q.C. Vuong:** None.

Nanosymposium

117. Functional Mechanisms of Attention

Location: 150A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 117.06

Topic: F.01. Human Cognition and Behavior

Support: Marie Curie CIG ACCDECMEM

ERC Starting Grant MULTITASK

Title: Computational modeling of the neural and behavioral correlates of mind-wandering and meditation

Authors: *M. K. VAN VUGT[1], N. A. TAATGEN[2], D. E. MEYER[3];
[1]Fac. of Mathematics and Physical Sci. - ALICE, [2]Artificial Intelligence and Cognitive Engin., Univ. of Groningen, Groningen, Netherlands; [3]Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: There is increasing evidence that meditation affects human cognition, emotion, motivation, and behavior. For example, one important aspect of meditation is its modulation of activity in the brain's default-mode network and changes in people's propensity to mind-wander. Yet little formal theory has been developed about meditation's underlying mental processes and brain mechanisms. To better understand these fundamental phenomena, we have formulated a computational cognitive model of contemplative practice and mind-wandering for one particular type of focused-attention meditation. Our model, developed collaboratively with Buddhist

scholars and implemented in the ACT-R cognitive architecture, precisely characterizes basic information-processing operations involved in meditation. Computer simulations based on the model yield predictions about both aspects of meditators' behavior and patterns of neural activity. Specifically, distractions during meditation are treated as being driven by a "thought pump" that primes haphazard mind-wandering, a process for which the DLPFC and hippocampus are known to be crucial. According to the model, (1) people repeatedly become distracted during meditation through failures to check where their attention is focused, and (2) eventual refocusing on the primary target object of attention occurs through reaccessing a symbolic representation of the intention to meditate stored in episodic memory. The present report summarizes initial tests of some of the model's basic predictions during a task that lets us track epochs of mind-wandering and manifests activities of the hypothesized thought pump. Our results provide first steps toward a firm theoretical and computational foundation for understanding the mental and physical substrates of meditation, which subsequently will allow us to make predictions about transfer between meditation practice and performance of other cognitive tasks.

Disclosures: **M.K. van Vugt:** None. **N.A. Taatgen:** None. **D.E. Meyer:** None.

Nanosymposium

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Location: 150A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 117.07

Topic: F.01. Human Cognition and Behavior

Title: Directed functional connectivity of prestimulus alpha relates to performance in a driving task

Authors: *A. D. PASSARO[1], J. BROOKS[2], J. VETTEL[3], S. KERICK[3], P. FRANASZCZUK[3]; [1]DCS Corp, Baltimore, MD; [2]Univ. of Maryland Med. Ctr., Baltimore, MD; [3]US Army Res. Lab., Aberdeen, MD

Abstract: Recent advancements in the domain of functional connectivity provide novel insight into the understanding of time-evolving brain networks that underlie changes in task performance. In this study, we use a directed connectivity measure derived from Granger causality, the directed transfer function (DTF), to examine relationships among functional connectivity and driving performance. In a driving simulator, subjects were told to drive along a long, monotonous road in a virtual environment while maintaining their vehicle centered in the

lane. Additionally, they were asked to quickly correct heading errors caused by simulated wind gusts (lateral perturbations to the vehicle). Previous studies have linked pre-stimulus alpha power to subsequent decrements in behavioral performance, and we extended this finding by analyzing directed functional networks of alpha power at the scalp level prior to the trial (perturbation onset). Trials with greater heading errors identified significant connections originating from left posterior regions on the scalp to both ipsilateral and contralateral anterior regions as compared to trials with smaller heading errors. Furthermore, a linear regression analysis found a positive relationship between heading error and the DTF connectivity values from connections emanating from posterior to anterior regions. These results indicate the potential for functional connectivity metrics to predict decrements in behavioral performance; in this case, greater posterior to anterior connections prior to trial onset corresponded to greater heading errors.

Disclosures: **A.D. Passaro:** None. **J. Brooks:** None. **J. Vettel:** None. **S. Kerick:** None. **P. Franaszczuk:** None.

Nanosymposium

117. Functional Mechanisms of Attention

Location: 150A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 117.08

Topic: F.01. Human Cognition and Behavior

Support: ERC Grant ERC-2010-StG-20091209

DFG Grant KE1828/2-1

Title: Intersensory attention and temporal orientation are reflected in distinct patterns of cortical oscillations and neural connectivity

Authors: *J. KEIL, U. POMPER, D. SENKOWSKI;
AG Multisensory Integration, Charité Med. Sch., Berlin, Germany

Abstract: Knowledge about the sensory modality in which a forthcoming event occurs modulates intersensory attention (IA). Information on when an event occurs enables temporal orienting (TO). Both attention mechanisms - IA and TO - can facilitate sensory stimulus processing. Previous studies have investigated the neural mechanisms underlying IA and TO separately and so far, it is unknown whether and how they may interact. In this EEG study we presented a continuous stream of temporally and spatially aligned visuo-tactile stimuli that were

preceded by an auditory cue. Tactile inputs were delivered to the left hand. Participants were instructed to respond to occasional bimodal targets by a button-press with their right hand. To manipulate IA, we used an auditory cue indicating to which modality participants had to attend (visual or tactile). We manipulated TO by presenting stimuli block-wise either at fixed or variable inter-stimulus intervals. Single trial EEG data were projected into source space using spatial filtering. We analyzed power, phase, and neural connectivity of time-frequency transformed data. Moreover, we computed graph theoretical measures to identify local and global networks underlying IA and TO. Irrespective of the cued modality, reaction times were faster when stimuli were presented with fixed compared to variable inter-stimulus intervals, demonstrating a facilitating effect of TO. This effect was reflected by increased suppression of beta-band (13-30 Hz) power in bilateral sensorimotor cortices. TO also modulated the power and intertrial coherence of the delta-band (0.5-4 Hz) in the left sensorimotor cortex. The effect of IA was reflected by increased suppression of beta-band power in the right sensorimotor cortex and increased suppression of beta- and alpha-band (8-12 Hz) power in the visual cortex. Intertrial coherence as well as local and global network measures revealed distinct patterns of IA and TO effects. Our study demonstrates that power, phase, and connectivity of neural oscillations in distributed cortical networks differentially reflect IA and TO. Our study also suggests that IA and TO can operate in parallel and in a widely independent manner.

Disclosures: J. Keil: None. **U. Pomper:** None. **D. Senkowski:** None.

Nanosymposium

117. Functional Mechanisms of Attention

Location: 150A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 117.09

Topic: F.01. Human Cognition and Behavior

Support: NSERC Postdoctoral Fellowship

Army Research Office Grant W911NF

Title: Visual selective attention is driven by competition between task-relevant and task-irrelevant features at early stages of visual processing

Authors: *M. MACLEAN[1], B. GIESBRECHT[1,2];
[1]Inst. for Collaborative Biotechnologies, Santa Barbara, CA; [2]Psychological & Brain Sci., Univ. of California Santa Barbara, Santa Barbara, CA

Abstract: A wealth of evidence demonstrates that task relevant and salient features influence selective attention. In a series of experiments we explored how task irrelevant, non-salient features drive visual selection, and modulate the influence of currently task relevant features. Participants (N=43, 3 experiments) performed a value learning task in which particular colors (red/blue) were associated with particular magnitudes of reward (5¢/1¢). One week after completing this task, the value conditioned features were included in a new search task, but were neither task relevant nor salient. Importantly, the value conditioned features either surrounded a task relevant target or a task irrelevant distracter that was either presented at a task relevant or irrelevant location. Compared to performance in a baseline condition in which there was no value conditioned feature (69%), there was a benefit to target identification accuracy when the value conditioned color was presented at the target location (76%, $p < 0.001$) and a cost when the color was presented at other locations (67%, $p < 0.01$). Effects were larger for high value features than low value ones (5% vs. 1%, $p < 0.001$). Thus, irrelevant value conditioned features influenced performance, resulting in both costs and benefits. We also observed that performance was influenced by an interaction of task relevant and irrelevant features. The effect of the value conditioned feature was larger when associated with a target (task relevant) than a distracter (task irrelevant; 5% vs. 1%, $p < 0.001$). The influence of the value conditioned feature was not significant when presented at a task irrelevant location (0.5%, p's = .466), indicating an interaction with task relevance in terms of space as well as identity. To investigate the stage of visual processing modulated by irrelevant value conditioned features we conducted an EEG study (n=15) where we measured the amplitude of the P1 ERP component within the first 200 ms of stimulus presentation. The P1 was larger at sites contralateral to the location of the value conditioned feature than at ipsilateral sites (6 μV vs. 5μV, $p < 0.001$), indicating that attentional selection was modulated by the irrelevant value conditioned feature at an early stage of visual processing. This effect was larger for high value features than low value ones, but only when associated with a target, indicating that the interaction of task relevant and irrelevant features is also present at early stages of visual processing. These results indicate that irrelevant non-salient features enhance spatial visual selection within the first few hundred milliseconds of processing, and do so as a function of task relevance.

Disclosures: **M. Maclean:** None. **B. Giesbrecht:** None.

Nanosymposium

117. Functional Mechanisms of Attention

Location: 150A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 117.10

Topic: F.01. Human Cognition and Behavior

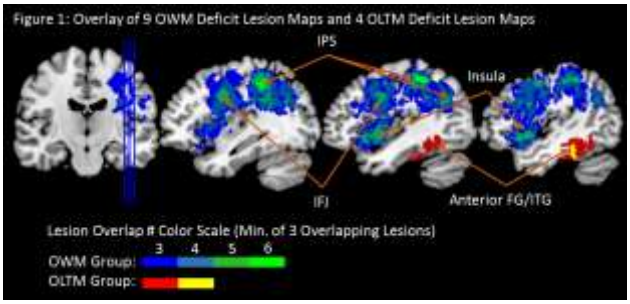
Support: NIH Grant DC006740

NIH Grant 1-P50-DC012283-01A1

Title: Separate neuroanatomical correlates for orthographic working memory and orthographic long term memory

Authors: *J. J. PURCELL[1], R. CAPASSO[2], G. MICELI[3], B. RAPP[1];
[1]Johns Hopkins Univ., Baltimore, MD; [2]IRCCS Fondazione Santa Lucia and SCA Associates, Roma, Italy; [3]Univ. di Trento, Trento, Italy

Abstract: In the orthographic domain, evidence for the distinction between the orthographic working memory (OWM) and orthographic long term memory (OLTM) has come primarily from studies of acquired dysgraphia subsequent to brain injury (e.g, Buchwald & Rapp, 2009). However, the neural bases of OWM and OLTM have not been extensively examined. Here we present findings from the first combined evaluation of brain lesion overlap in a set of individuals with well-documented OWM and OLTM impairments. We identified the brain lesion volumes of 13 individuals with left-hemisphere damage, consisting of OWM and OLTM deficit groups. The OWM deficit group (9; 6 males) had OWM deficits in spelling, without orthographic lexical or sublexical deficits. The OLTM deficit group (4; 1 male) had OLTM deficits in spelling, without OWM deficits. Normalization was carried out in SPM8; lesion maps were overlaid on a template brain (geometric centers reported in MNI coordinates). This analysis revealed a single region in the left anterior inferior temporal/fusiform gyrus (ant-FG/ITG) associated with OLTM that was clearly distinct from a set of frontal-parietal areas associated with OWM (see Figure 1). The lesions of all 4 of the OLTM participants overlapped in the left ant-FG/ITG region (-51, -42, -19). In the OWM group there were 3 areas of high density lesion overlap: (1) along the intraparietal sulcus (IPS), with an anterior concentration (-36, -49, 39); (2) the inferior frontal junction (IFJ;-44, -11, 38) and (3) the insula (-43, 5, 0). First, these results revealed a clear distinction in the neurotopography of OWM and OLTM lesion sites that is well-aligned with neuroimaging results in neurally intact participants (e.g., Rapp & Dufor, 2011). Second, these neurotopographic differences support the claims of the computational distinctiveness of long-term vs. working memory operations. Finally, the multiple specific lesion loci for OWM raise the possibility that different lesion sub-regions may correspond to different components of the OWM system.



Disclosures: **J.J. Purcell:** None. **R. Capasso:** None. **G. Miceli:** None. **B. Rapp:** None.

Nanosymposium

117. Functional Mechanisms of Attention

Location: 150A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 117.11

Topic: F.01. Human Cognition and Behavior

Title: Speaker identity judgments in humans can be predicted by patterns of hemodynamic activity in the right Temporal Voice Area

Authors: ***M. WOLMETZ**[1], A. M. LEAVER[2], M. A. CHEVILLET[1];
[1]Johns Hopkins Applied Physics Lab., Laurel, MD; [2]Dept. of Neurol., Ahmason-Lovelace Brain Mapping Center, UCLA, Los Angeles, CA

Abstract: In verbal communication, the speaker's identity, message, and affect are all encoded in the acoustic speech signal, and listeners are remarkably successful at decoding all three types of information. In some situations, however, we fail: words are misperceived, speakers are misidentified, and emotions are misread. In this study, we sought to better understand how such errors arise in the human auditory system by analyzing the hemodynamic responses evoked when human subjects made difficult speaker identification judgments. Using a multivariate pattern analysis approach, we probed the degree to which speaker identity could be decoded from the patterns of activity present in different brain regions implicated in speaker identity judgments. Functional Magnetic Resonance Imaging (fMRI) data were collected for twelve participants while they performed a one-back speaker identity discrimination task on short spoken phrases. Behavioral results both in and out of the scanner indicated that the voice pairs presented were highly confusable, i.e., performance was poor but significantly above chance levels. The neural responses were then used to decode the identities of the speakers present

across trials. Decoding accuracy for activity patterns in the right (but not left) Temporal Voice Area (TVA) region of interest was significantly correlated with listener performance. Several previous studies support a role for right hemisphere TVA in speaker identity judgments, and our results further suggest that activity in the right TVA may be responsible for driving the behavioral response. A subsequent whole-brain searchlight analysis demonstrated that amygdala activity was the best predictor of how listeners should have responded (rather than how they actually, often incorrectly, responded). Our results suggest that amygdala-encoded information may be beneficial but underused when making difficult speaker identity judgments, consistent with reports linking amygdala response to both affective processing and speaker identity, and behavioral findings linking affective vocal characteristics to speaker recognition.

Disclosures: **M. Wolmetz:** None. **A.M. Leaver:** None. **M.A. Chevillet:** None.

Nanosymposium

117. Functional Mechanisms of Attention

Location: 150A

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 117.12

Topic: F.01. Human Cognition and Behavior

Support: 5R01AG019731-11

Title: The Conditions of Cross-Hemispheric Communication: The role of structural and functional connectivity in mediating perceptual and conceptual processing

Authors: *S. W. DAVIS, R. CABEZA;
Duke Univ., Durham, NC

Abstract: A rich body of research supports the general idea that the left and right cerebral hemispheres are specialized for specific cognitive operations: the left hemisphere for language and verbal material, the right hemisphere for holistic and spatial processing. These asymmetries, however, are rooted in a processing system with a high degree of overall similarity between the hemispheres, both in how information is represented and processed, as well as how these complementary regions interact to perform meaningful cognitive operations. Nonetheless, the neural basis of interhemispheric interaction (IHI) is a relatively unexplored phenomenon, and the regional specificity of IHI during cognition is unmapped. In the current experiment we tested the role of both task difficulty and domain of processing (either semantic matching of words or perceptual matching of faces) in mediating the expression of and reliance on structural and

functional connectivity. We found both structural and functional correlates of IHI. First, subjects performed better on bilaterally presented trials as task difficulty increased, in both the semantic and perceptual matching tasks. Second, correlations between the speed advantage for bilaterally presented pairs and FA in the corpus callosum were more pronounced in anterior callosum for semantic matching, and while the advantage for bilaterally presented face pairs was correlated with FA of the posterior callosum. Functional connectivity, on the other hand, exhibited both domain-specific and domain-general effects, such that fCON decreased within homotopic regions with increasing difficulty in domain-specific regions (semantic matching: temporal pole; perceptual matching: fusiform gyrus), and increased with increasing difficulty in anterior frontal cortex. These findings clarify the mechanisms by which the hemispheres interact to perform complex cognitive tasks, and suggest a domain-general role of anterior frontal cortex in mediating successful performance in semantic and face processing.

Disclosures: S.W. Davis: None. **R. Cabeza:** None.

Nanosymposium

118. Attentional Networks in Humans

Location: 147A

Time: Sunday, November 16, 2014, 8:00 AM - 10:30 AM

Presentation Number: 118.01

Topic: F.01. Human Cognition and Behavior

Support: NIH R01EY022229

Title: Frontal networks for visual and auditory attention: Mining functional connectivity in the Human Connectome Project

Authors: *S. M. TOBYNE[1], D. E. OSHER[2], S. W. MICHALKA[1,3], D. C. SOMERS[2,1,3]; [1]Grad. Program for Neurosci., [2]Psychological and Brain Sci., Boston Univ., Boston, MA; [3]Ctr. for Computat. Neurosci. and Neural Technol. (CompNet), Boston, MA

Abstract: Recent work in our laboratory has suggested that human caudal lateral frontal cortex contains four interleaved regions in each hemisphere that exhibit strong sensory-specific biases in attention tasks (Michalka et al, 2014). Two auditory-biased attention regions, caudal inferior frontal sulcus (cIFS) and the transverse gyrus intersection the precentral sulcus (tgPCS), anatomically alternate with two visual-biased attention regions, superior and inferior pre-central sulcus (sPCS, iPCS). These small regions were identified in fMRI studies in a small number of individual subjects. Here, we have investigated these regions and their putative networks by

mining the WashU-Minn Human Connectome Project (HCP) dataset. Data was used from 86 HCP participants for whom both diffusion-weighted imaging and resting-state fMRI are available. This abstract reports the results of resting-state functional connectivity analysis. To perform our analysis, we defined seed regions from our individual subject task fMRI data that contrasted auditory and visual spatial attention. Statistical activation maps were coregistered and thresholded to generate probabilistic regions of interest (ROIs). These ROIs served as seed regions for analysis of the HCP dataset. Stronger functional connectivity was observed for the sPCS and iPCS than for tgPCS and cIFS with superior parietal lobule visual attention regions, and conversely stronger connectivity was observed for the tgPCS and cIFS than for sPCS and iPCS with superior temporal lobe auditory attention regions. This supports our prior claims of interleaved auditory-visual organization in lateral frontal cortex. A long-term goal of this analysis is to develop reliable methods for identifying fine scale brain networks in large population datasets, which could have important clinical application. Our preliminary results reveal both successes and challenges of these efforts.

Disclosures: S.M. Tobyne: None. **D.E. Osher:** None. **S.W. Michalka:** None. **D.C. Somers:** None.

Nanosymposium

118. Attentional Networks in Humans

Location: 147A

Time: Sunday, November 16, 2014, 8:00 AM - 10:30 AM

Presentation Number: 118.02

Topic: F.01. Human Cognition and Behavior

Support: NIH R01EY022229

Title: Frontal networks for visual and auditory attention: Mining structural connectivity in the Human Connectome Project

Authors: *D. E. OSHER[1], S. M. TOBYNE[2], S. W. MICHALKA[2,3], D. C. SOMERS[1,2,3]; [1]Psychological & Brain Sci., [2]Grad. Program for Neurosci., [3]Ctr. for Computat. Neurosci. and Neural Technol. (CompNet), Boston Univ., Boston, MA

Abstract: Recent work in our laboratory has suggested that human caudal lateral frontal cortex contains four interleaved regions in each hemisphere that exhibit strong sensory-specific biases in attention tasks (Michalka et al, 2014). Two auditory-biased attention regions, caudal inferior frontal sulcus (cIFS) and the transverse gyrus intersection the precentral sulcus (tgPCS),

anatomically alternate with two visual-biased attention regions, superior and inferior pre-central sulcus (sPCS, iPCS). These small regions were identified in fMRI studies in a small number of individual subjects. Here, we have investigated these regions and their putative networks by mining the WashU-Minn Human Connectome Project (HCP) dataset. Data was used from 86 HCP participants for whom both diffusion-weighted imaging and resting-state fMRI are available. This abstract reports the tractography results. To perform our analysis, we defined seed regions from our individual subject data in a task that contrasted auditory and visual spatial attention. Probabilistic activation maps were constructed and then thresholded to generate ROIs. These ROIs served as seed regions for tractography analysis of the HCP dataset. The tractography results reveal complimentary anatomical gradients of connectivity with the more dorsal regions (sPCS, tgPCS) exhibiting the highest probabilities of connection to the parietal ROI and the more ventral regions (cIFS, iPCS) exhibiting the highest probabilities of connection to the temporal ROI. This differs from the interleaved auditory-visual organization in lateral frontal cortex observed in task-based fMRI and resting-state functional connectivity. A long-term goal of this analysis is to develop reliable methods for identifying fine scale brain networks in large population datasets, which could have important clinical application. Our preliminary results reveal both successes and challenges of these efforts.

Disclosures: **D.E. Osher:** None. **S.M. Tobyne:** None. **S.W. Michalka:** None. **D.C. Somers:** None.

Nanosymposium

118. Attentional Networks in Humans

Location: 147A

Time: Sunday, November 16, 2014, 8:00 AM - 10:30 AM

Presentation Number: 118.03

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant EY021644

NSF Grant BCS-1059523

Title: Sensory processing with varying degrees of attention - Lessons from parietal lobe damage

Authors: *S. S. SHOMSTEIN[1], F. UYAR[2], A. S. GREENBERG[3], M. BEHRMANN[4]; [1]Psychology and Inst. for Neurosci., George Washington Univ., Washington, DC; [2]Univ. of Pittsburgh, Pittsburgh, PA; [3]Univ. of Wisconsin - Milwaukee, Milwaukee, WI; [4]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Following right parietal lobe damage, patients with hemispatial neglect display impairments in attending to information on the left side of space. Recent theories suggest that this spatial attention deficit arises from structural/functional perturbation of the balance between the dorsal and ventral attentional networks. The consequence of the attentional imbalance on the sensory signals elicited in response to visual stimulation is not yet understood. Neglect offers a unique opportunity to examine the direct consequences of attention on sensory processing by measuring behavioral and neural responses to a stimulus presented in the unaffected right side of space compared with the same stimulus presented to the affected left side of space. Here, we contrasted neural signals elicited in response to the attended and neglected visual stimulation in patients with right parietal lobe lesions and intact occipital and temporal cortex. FMRI was used to localize four visual field locations in sensory regions V1-V4, along with FFA and PPA. In addition, patients performed a fixation task while task-irrelevant images of faces and houses were presented in the targeted 4 locations. Univariate analysis showed greater difference between the preferred and non-preferred stimuli as one moves anteriorly from V1, V2, V3, V4, to PPA, and FFA, in the left but not in the right hemisphere. The reduction of right hemisphere response to preferred stimuli was correlated with the severity of neglect. The signal integrity in the two hemispheres was tested separately with multivariate analysis. Category and location information was degraded in the right versus the left hemisphere, as evidenced by lower within-category cross-correlation coefficients and classification accuracies. These results provide evidence that attention directly affects perceptual processing by improving the integrity of sensory responses elicited throughout visual cortex and by magnifying the difference between responses to preferred and non-preferred stimuli.

Disclosures: S.S. Shomstein: None. **F. Uyar:** None. **A.S. Greenberg:** None. **M. Behrmann:** None.

Nanosymposium

118. Attentional Networks in Humans

Location: 147A

Time: Sunday, November 16, 2014, 8:00 AM - 10:30 AM

Presentation Number: 118.04

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH086709

Title: The strength of a pupil-associated resting-state functional network relates to trait-level attention

Authors: *A. BREEDEN[1], M. NORR[2], G. SIEGLE[3], C. J. VAIDYA[1];
[1]Georgetown Univ., Washington, DC; [2]Univ. of California, Berkeley, Berkeley, CA; [3]Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA

Abstract: The ability to sustain attention or maintain arousal in the absence of external stimulation is reduced in individuals with Attention Deficit Hyperactivity Disorder (ADHD). Pupil diameter is often used as an index of arousal and cognitive processing and has been recently associated with a frontal-parietal network (Murphy et al., 2014). Whether this pupil-related network differs across individuals varying in the ability to sustain attention is unknown. Here we delineated the functional network associated with pupil diameter during a task-free resting state and examined whether it predicted individual differences in attentional function in everyday life. Fifteen 18-27 year old adults were scanned at rest for 12 minutes with controlled (low) room and screen luminance. Functional images were slice time corrected, realigned, normalized and smoothed. Pupil diameter was recorded at 60 Hz during functional image acquisition with an MRI-integrated infrared camera. Pupil data were preprocessed using methods described in (Siegle et al., 2003), including: noise and blink correction by interpolating through time points which displayed a greater than expected rate of change, high pass (128 Hz) filtering, and convolving pupillary time courses with a hemodynamic response function. First-level statistical maps relating resting brain activity to fluctuations in pupil diameter were created for each participant by modeling fMRI activity in each voxel as a function of pupil diameter, controlling for head motion, and mean white matter and CSF signal. The relationship between attentional traits and pupil-brain coupling was assessed by correlating the mean strength of each participant's first-level pupil map with self-reported inattentiveness in every-day life measured by the Adult ADHD Self-Report Scale. Pupil diameter was significantly associated with resting activity in a cluster of attention related regions, including bilateral anterior insula, bilateral superior frontal gyrus, right inferior parietal lobe, anterior cingulate, and middle cingulate. Across participants, there was a significant negative correlation (r = -.68) between mean pupil-brain coupling and trait level inattentiveness. Pupil diameter was significantly related to resting brain activity in a broad network of attention and autonomic-control related regions. Moreover, the strength of this relationship predicted trait-level inattentiveness. This suggests that the status of coordinated activity between attentional brain areas and autonomic systems controlling pupil diameter during a task-free state contributes to phenotypic attentional differences.

Disclosures: **A. Breeden:** None. **M. Norr:** None. **G. Siegle:** None. **C.J. Vaidya:** None.

Nanosymposium

118. Attentional Networks in Humans

Location: 147A

Time: Sunday, November 16, 2014, 8:00 AM - 10:30 AM

Presentation Number: 118.05

Topic: F.01. Human Cognition and Behavior

Support: NIH-NIDCD P50 DC000422

NIH-NCRR UL1 RR029882

NIH-NCRR C06 RR14516

Title: The cingulo-opercular network supports word recognition in noise for older adults

Authors: *K. VADEN, JR, S. E. KUCHINSKY, S. L. CUTE, J. B. AHLSTROM, J. R. DUBNO, M. A. ECKERT;
Otolaryngology-Head and Neck Surgery, Med. Univ. of South Carolina, Charleston, SC

Abstract: Speech recognition in noise can be particularly challenging for older adults and elicits activity in a domain-general cingulo-opercular network that is thought to monitor performance and facilitate adaptive control. We tested a long-standing hypothesis that the cingulo-opercular network supports performance in older adults using a word recognition task. Healthy older adults (N=31; 50-81 years of age; mean pure tone thresholds < 28 dB HL from 0.25-8 kHz) performed word recognition in multitalker babble at two signal to noise ratios (SNR=+3 dB or +10 dB) during a sparse sampling fMRI experiment. As expected, word recognition in babble was poorer in the +3 dB SNR condition (m=42.6%) compared to the +10 dB SNR condition (m=70.4%), but was not related to mean pure tone thresholds in quiet (r=-0.19, ns). Generalized linear mixed modeling demonstrated that elevated cingulo-opercular activity was significantly associated with an increased likelihood for correct word recognition on the next trial ($Z>2.26$, cluster extent FWE $p<0.05$), independent of preceding task performance and SNR condition. This predictive relationship was larger for participants with better mean performance ($r=0.53$, $p=0.002$) and did not change with age ($r=-0.12$, ns). Cuneus and left occipito-temporal activity also significantly related to correct word recognition on the next trial. Although elevated visual cortex activity occurred with elevated performance on a trial-by-trial basis, this association was strongest in older adults ($r=0.37$, $p=0.03$) with poorer overall performance ($r=-0.87$, $p<0.001$). Thus, in the context of adaptive control, our results support the premise that cingulo-opercular activity provides a speech recognition benefit for older adults.

Disclosures: K. Vaden: None. **S.E. Kuchinsky:** None. **S.L. Cute:** None. **J.B. Ahlstrom:** None. **J.R. Dubno:** None. **M.A. Eckert:** None.

Nanosymposium

118. Attentional Networks in Humans

Location: 147A

Time: Sunday, November 16, 2014, 8:00 AM - 10:30 AM

Presentation Number: 118.06

Topic: F.01. Human Cognition and Behavior

Support: Harvard Center for Neurodegeneration and Repair

Title: Simultaneous TMS and fMRI to study brain connectivity, adaptive plasticity and therapeutic neuromodulation of attentional networks

Authors: *J. A. CAMPRODON[1], A. SACK[2], A. PASCUAL-LEONE[3];
[1]Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA; [2]Maastricht Univ., Maastricht, Netherlands; [3]Beth Israel Deaconess Med. Center, Harvard Med. Sch., Boston, MA

Abstract: BACKGROUND: Attention is a key cognitive function of relevance to numerous neuropsychiatric disorders. Our aim is to use the simultaneous combination of TMS-fMRI to explore the physiological connectivity of attentional networks and their intrinsic compensatory plastic mechanisms causally, in a model of focal parietal lesion and hemispatial neglect. METHOD: We studied 10 healthy subjects while they performed a spatial attention task in the scanner with an MRI-compatible TMS coil placed over the right parietal cortex. The task involved 5 different conditions during which subjects covertly attended to the left or right, attended to the left or right while receiving TMS, or received TMS at rest. We studied intrinsic compensatory strategies by comparing the "pathological condition" (attending under the influence of TMS) to the "healthy condition" (attending without TMS) and controlling for the effects of TMS at rest. RESULTS: We describe a bilateral pattern of activatory and inhibitory changes within the attentional network, revealing a circuit-wide adaptive strategy to maintain functional competence despite the "virtual" TMS lesion. The analysis of the areas of maximum significance identifies a contralesional focus of parietal inhibition, which suggests a possible therapeutic target for neglect rehabilitation in line with current neurorehabilitation protocols. CONCLUSIONS: These data reveal the potential of the simultaneous combination of TMS-fMRI to study connectivity and plasticity in a translational framework. We suggest pathophysiological mechanisms of functional recovery and a therapeutic roadmap for functional rehabilitation of structural lesions. This approach could be used to study other neuropsychiatric disorders, due to structural or functional lesions.

Disclosures: J.A. Camprodon: None. **A. Sack:** None. **A. Pascual-Leone:** None.

Nanosymposium

118. Attentional Networks in Humans

Location: 147A

Time: Sunday, November 16, 2014, 8:00 AM - 10:30 AM

Presentation Number: 118.07

Topic: F.01. Human Cognition and Behavior

Support: K Award - 10015105

Title: The role of intact right superior temporal gyrus in object-centered spatial processing

Authors: K. CAULFIELD[1], *P. P. SHAH[2], F. GERVITS[3], P. CHEN[4], R. H. HAMILTON[3]; [1]Inst. of Cognitive Neurosci., Univ. Col. London, London, United Kingdom; [2]LABORATORY FOR COGNITION AND NEURAL STIMULATION, UNIVERSITY OF PENNSYLVANIA, PHILADELPHIA, PA; [3]Dept. of Neurol., Univ. of Pennsylvania, Philadelphia, PA; [4]Stroke Res. Lab., Kessler Fndn., West Orange, NJ

Abstract: The objective of the current study was to identify the neural correlates of spatial processing in healthy individuals in body- and object-centered frames of references. Neuropsychological and functional neuroimaging research suggests that the right posterior parietal cortex is crucially involved in the processing of body-centered spatial coordinates. However, the role of the superior temporal gyrus (STG) is unclear. While damage in the right STG is associated with spatial neglect, its function in healthy individuals remains highly debated. Recent evidence using transcranial magnetic stimulation (TMS) suggests that the right STG is involved in feature-based spatial exploration in visual search tasks (Ellison et al., 2004). However, in another study, TMS over the right STG during a landmark judgment task did not produce the expected neglect-like rightward bias in spatial judgments (Oliveri and Vallar, 2009). In our study, we used an offline inhibitory repetitive TMS (rTMS) protocol to investigate the role of the right STG in landmark tasks with respect to object-centered reference frames. Eleven healthy, right-handed adults underwent a modified landmark task in which pre-marked horizontal lines were randomly presented across the left (L) and right (R) hemifields. After viewing each stimulus, subjects had to judge whether the pre-marked line was bisected (bisection), or whether the left (L-long) or right side (R-long) of the line was longer. Subjects repeated the task before and after receiving 20 minutes of 1 Hz rTMS over the right SMG, STG, and vertex (a control site) on 3 separate days. We predicted that the proportion of R-long errors (indicative of induced rightward bias) would increase in the L-hemifield after stimulation over the right SMG, while R-long errors would increase in both hemifields post-STG stimulation. As our pre-stimulation analyses showed that subjects had ceiling effects in the L-long and R-long conditions, only the bisection conditions were analyzed. A linear mixed effects model including fixed effects of

hemifield (L, R), stimulation site (STG, SMG, Vertex) and session (pre, post) and the subject- and hemifield-level random effects revealed that 2-way interactions between site and session ($F=5.83$; $p=0.004$), and hemifield and session ($F=4.78$; $p=0.031$) were significant, while the 3-way interaction was not significant ($p=0.425$). This increase in R-long errors after STG stimulation, but not SMG or vertex stimulation, is consistent with neglect-like rightward bias. Our findings suggest a causal role of the right STG in a modified landmark task and highlight its function in object-centered spatial processing.

Disclosures: K. Caulfield: None. **P.P. Shah:** None. **F. Gervits:** None. **P. Chen:** None. **R.H. Hamilton:** None.

Nanosymposium

118. Attentional Networks in Humans

Location: 147A

Time: Sunday, November 16, 2014, 8:00 AM - 10:30 AM

Presentation Number: 118.08

Topic: F.01. Human Cognition and Behavior

Title: Functional connectivity of the right temporoparietal junction is modulated by the relevance of unattended stimuli

Authors: *C. LYNCH[1], X. YOU[1], M. NORR[1], E. GORDON[1], C. VAIDYA[1,2];
[1]Georgetown Univ., Washington, DC; [2]Children's Natl. Med. Ctr., Washington, DC

Abstract: Adaptive behavior requires flexible reorienting of attention to unexpected but important stimuli. Such reorienting is thought to engage the ventral attention network (VAN). Corbetta and colleagues posit that activation of the right temporoparietal junction (rTPJ), a key VAN node, is limited to task-relevant stimuli regardless of sensory salience (SS). Building on this model, we hypothesized that the functional connectivity of the rTPJ is also selectively modulated by task-relevance. We tested our hypothesis by designing two runs of a selective attention task that required subjects to respond to a centrally presented target triangle. In the first run, unexpected distractors, of either low or high SS, appeared in the periphery and were irrelevant (Run1: IR). In the second run, in addition to the central task subjects were instructed to respond to a high SS task-relevant distractor (Run2: TR). Thus, the runs were identical, except for the relevance of distractor stimuli. Fifty healthy adults completed both fMRI runs on a 3T Siemens Tim Trio and the Adult ADHD Self-Report Scale (ASRS). Data were preprocessed and analyzed using SPM8. Activation of the rTPJ to high vs. low SS distractors was assessed using a general linear model (GLM) and compared between runs using a paired t-test. To measure rTPJ

connectivity differences between runs, task conditions were regressed out and rTPJ seed-based "residual" functional connectivity (rFC) maps were generated for each subject. Differences in rFC between runs were assessed using a paired t-test ($p<0.05$, monte carlo corrected). A step-wise multiple regression analysis tested if rTPJ connectivity to regions identified by the paired t-test predicted rTPJ activation to task-relevant distractors and ASRS scores. We report three main results. First, GLM analyses confirmed rTPJ activation is limited to task-relevance. In response to high SS distractors the rTPJ was suppressed in the IR run and activated in the TR run ($p<0.01$). Second, rTPJ rFC increased with bilateral posterior parietal cortex (PPC) and dorsolateral prefrontal cortex in the TR run relative to the IR run. In contrast, rFC was increased within the VAN, including bilateral anterior insula and right inferior frontal cortex in the IR run relative to the TR run. Finally, modulation of rTPJ-PPC connection predicted rTPJ activation in the TR run ($r = 0.37$, $p<0.01$) and ASRS attentional impulsivity scores ($r = -0.38$, $p<0.008$). While rTPJ activation findings are in line with Corbetta's model, our rFC findings extend this theoretical framework by demonstrating that rFC of the rTPJ is also sensitive to relevance and that this is behaviorally meaningful.

Disclosures: **C. Lynch:** None. **X. You:** None. **M. Norr:** None. **E. Gordon:** None. **C. Vaidya:** None.

Nanosymposium

118. Attentional Networks in Humans

Location: 147A

Time: Sunday, November 16, 2014, 8:00 AM - 10:30 AM

Presentation Number: 118.09

Topic: F.01. Human Cognition and Behavior

Support: NIH 2T32MH065214-11

Title: Topographic subunits of the attentional control network are differentially modulated by cue validity

Authors: ***M. SCOLARI**, S. KASTNER;
Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: There are 18 topographic subunits within fronto-parietal cortex that are known to direct the locus of top-down attention by generating weights in favor of the contralateral visual field. Previous research has broadly implicated this region in top-down attentional reorienting between a high-probable precued location to a low-probable uncued location (Vossel et al.,

2006). Given that the network was defined anatomically, however, it remains unclear how each region individually contributes to the complex process of selection during tasks that require top-down attentional reorienting under a range of cuing probabilities. Here we use high resolution fMRI to investigate how different subunits of the control network signal parametric fluctuations in the behavioral relevance of a stimulus by manipulating the validity of a spatial pre-cue. During each 12s stimulus block, gratings flickered at a rate of 10Hz on both sides of fixation. A central pre-cue indicated whether targets would appear within the left or right stimulus with a pre-specified probability level (50%, 75% or 100% valid), giving five levels of probabilities for each location (0%, 25%, 50%, 75% and 100%). Univariate analyses revealed that control subunits were differentially modulated by cue validity: while activation within IPS0 monotonically increased across all probabilities, IPS3-5 (aIPS) and FEF exhibited peaks of activation when the pre-cue was 75% valid. These results suggest that IPS0 signals the amount of attention directed to a location, whereas aIPS and FEF are likely involved in attentional disengagement from a high-probable cued location to a low-probable uncued location. Next, multivariate pattern analyses were used to investigate temporal fluctuations of spatial selection in these subunits across each of the probability conditions. Classification evidence-- an estimate of the correspondence between neural activation and each category it was trained on-- was plotted in order to infer which of the two gratings was the locus of selection for each 2s TR. The results suggest that when both locations are behaviorally relevant, attentional control slowly fluctuates between them within the network, such that one is favorably selected at the expense of the other. Furthermore, relative to missed targets, correctly detected targets in the 50% condition were associated with greater evidence for the cued location in IPS0. This study provides new and important evidence about the way in which subunits differentially operate during space-based selection.

Disclosures: **M. Scolari:** None. **S. Kastner:** None.

Nanosymposium

118. Attentional Networks in Humans

Location: 147A

Time: Sunday, November 16, 2014, 8:00 AM - 10:30 AM

Presentation Number: 118.10

Topic: F.01. Human Cognition and Behavior

Support: WE 4299/3-1

Title: The role of visuo-spatial attention in bottom-up salience coding

Authors: *R. WEIDNER[1], S. BERTLEFF[1], G. R. FINK[1,2];
[1]Cognitive Neurosci., Res. Ctr. Juelich, Juelich, Germany; [2]Univ. Hosp. Cologne, Cologne, Germany

Abstract: Attentional selection is either guided by specific features of a sensory stimulus (bottom-up) and/or by internal settings of the observer (top-down). There is an ongoing debate on how these two processes interact. Particularly, there is controversial evidence on whether or not salient but task irrelevant visual stimuli (distractors) can be ignored based on top-down control settings (Theeuwes, 1992; Bacon & Egeth, 1994). In order to account for these apparently ambiguous findings, Theeuwes (2004) emphasized the role of spatial attention suggesting that salience calculation occurs within but not outside the spatial focus of attention. Accordingly, only a salient item within this attentional window will capture attention automatically whereas stimuli outside the spatial focus can be ignored deliberately. The current study investigated the role of visuo-spatial attention in calculating salience using functional magnetic resonance imaging (fMRI) (n = 27) and a variant of the irrelevant distractor paradigm (Theeuwes, 1992). The size of the spatial attentional spotlight was experimentally varied by either presenting a perfectly valid cue (100 %) hence generating a small attentional focus centered at the target location or, alternatively, a spatially unpredictive cue. Behaviorally, a small attentional spotlight abolished the irrelevant distractor effect (distractor presence vs. absence: 7 msec) observed with unpredictive cues (distractor presence vs. absence: 26 msec). To test whether the missing irrelevant distractor effect was based on an altered coding of distractor salience, in addition to the main fMRI experiment functional position localizer scans were performed. This procedure allowed extracting estimates of BOLD-amplitudes for each experimental condition at specific retinotopic locations. Overall, irrelevant distractors enhanced BOLD signals at their respective retinotopic representations (main effect distractor (presence vs. absence): $F(1, 27) = 5.296$, $p < .05$). However, this increase induced by distractor presence was unaffected by the size of the attentional spotlight (no interaction of distractor (presence vs. absence) and attentional focus (small vs. wide): $F(1, 27) = 0.379$, $p =$ n. s.), suggesting that distractor salience is also coded outside the spotlight of attention. References Bacon, W. F., & Egeth, H. E. (1994). Overriding stimulus-driven attentional capture. Percept & Psychophys, 55(5), 485-96. Theeuwes, J. (1992). Perceptual selectivity for color and form. Percept & Psychophys, 51(6), 599-606. Theeuwes, J. (2004). Top-down search strategies cannot override attentional capture. Psychonom Bullet & Rev, 11(1), 65−70.

Disclosures: R. Weidner: None. **S. Bertleff:** None. **G.R. Fink:** None.

Nanosymposium

119. Corticolimbic Circuits and Decision-Making

Location: 206

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 119.01

Topic: F.02. Animal Cognition and Behavior

Support: CFI 14033

CIHR 102507

Title: Decision making requires interaction of the nucleus accumbens with ventral hippocampus, not with orbitofrontal cortex

Authors: *A. R. ABELA, Y. CHUDASAMA;
Psychology, McGill Univ., Montreal, QC, Canada

Abstract: Several lines of evidence suggest that the nucleus accumbens (NAcc) and orbitofrontal cortex (OFC) work together to enable optimal decision making, especially in the face of risk and uncertainty. For example, rats with NAcc or OFC lesions cause rats to be 'risk averse,' biasing their choices toward safe, small rewards, rather than large rewards that are uncertain (Cardinal and Howes, 2005; Abela and Chudasama, 2013). The NAcc presumably interacts with the ventral hippocampus (vHC), since lesions to either structure make rats 'delay averse' leading to impulsive choices. Both the OFC and vHC project to the NAcc (Groenewegen et al., 1987; 1997) where they converge with dopaminergic input arising from the ventral tegmental area, which acts as an important modulator of motivated behaviour (Salamone et al., 2005; Floresco et al 2008). Thus, both OFC and vHC projections are thought to be important determinants of NAcc activity. This prompted us to use a disconnection lesion procedure to understand how these structures are concurrently engaged in decision making. Rats received a lesion of the NAcc in one hemisphere combined with either a vHC or OFC lesion in the same hemisphere ("ipsilateral") or in the opposite hemisphere ("disconnection"). Rats were then tested on two decision-making tasks. In delay discounting, rats chose between two visual stimuli, where one stimulus delivered a small, immediate reward and the other a large delayed reward. In probability discounting, a new pair of visual stimuli indicated instead a small, certain reward or a large, uncertain reward. All animals, irrespective of group, preferred the large reward when it was delivered immediately, and shifted their preference to the small, immediate reward as the delay to the large reward increased. However, only those animals with the vHC-NAcc disconnection were intolerant of delay showing a strong bias toward the small, immediate reward. Surprisingly, none of the groups, including those with OFC-NAcc disconnections were affected by changes in reward probability suggesting that although the NAcc and the OFC are both necessary, their interaction is not sufficient for the decision making process. Instead, we demonstrate that making decisions with delayed outcomes requires the necessary interaction of the vHC and NAcc.

Disclosures: **A.R. Abela:** None. **Y. Chudasama:** None.

Nanosymposium

119. Corticolimbic Circuits and Decision-Making

Location: 206

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 119.02

Topic: F.02. Animal Cognition and Behavior

Support: NSERC Discovery Grant 402642

Title: The ventral hippocampus is critical for approach-avoidance conflict resolution: Introducing a novel rodent paradigm

Authors: ***R. ITO**, A. SCHUMACHER;
Psychology, Univ. of Toronto Scarborough, Toronto, ON, Canada

Abstract: 'To approach, or not to approach?' The effective resolution of a conflict between two such incentive motivations (approach vs. avoidance) is paramount in our ability to make day-to-day decisions, and involves a complex computation of the value, likelihood and magnitude of the outcomes and incentive stimuli associated with the outcomes. Much research has been directed at delineating the neural circuitry underlying approach motivation and avoidance motivation separately, implicating the amygdala-medial prefrontal cortex-striatal pathway as being critical. Very little research, however, has directly examined the neural circuits that are engaged when opposing incentive motivations are experienced simultaneously. We hereby present a novel approach-avoidance paradigm that directly measures a state of motivational conflict induced by the simultaneous presentation of cues which predict both appetitive and aversive outcomes. Furthermore, this study sought to examine the role of the dorsal and ventral hippocampus (HC) in approach-avoidance conflict resolution, revisiting a once popular theory of HC function which posited the HC to be the driving force of a behavioural inhibition system that is activated in situations of imminent threat to inhibit an action that could be detrimental to survival. It was hypothesized, based on recent evidence indicating the ventral HC to be important in inhibitory control, that the ventral, but not dorsal HC is important in the control of approach/avoidance motivational influences upon behavior. Male Long Evans rats received pre-training excitotoxic lesions (NMDA) of the dorsal or ventral HC, and were subsequently trained in a concurrent mixed valence conditioning paradigm to associate different non-spatial cues with rewarded, aversive and neutral outcomes, using three arms of a radial maze apparatus. On the final day of testing, a state of approach-avoidance conflict was induced by superimposing two stimuli of

opposite valences in one arm, and comparing the time the rats spent interacting with the superimposed 'conflict' cue, as opposed to the neutral cue. All rats showed successful acquisition of the mixed valence conditioning. Furthermore, the ventral HC lesioned group spent significantly more time with the conflict cue than the neutral cue, compared to the dorsal HC-lesioned, and control groups. Thus, we provide evidence that the ventral, but not dorsal HC, is a crucial component of the neural circuitry concerned with conflict resolution. In its absence, approach tendencies are potentiated, which would indicate that under normal circumstance, it serves to exert inhibitory control over approach tendencies.

Disclosures: R. Ito: None. **A. Schumacher:** None.

Nanosymposium

119. Corticolimbic Circuits and Decision-Making

Location: 206

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 119.03

Topic: F.02. Animal Cognition and Behavior

Support: CIHR

Title: Bilateral prefrontal traumatic brain injury causes severity-dependent, chronic deficits in attention, motivation, motor impulsivity and response latency in rats

Authors: *C. VONDER HAAR, C. A. WINSTANLEY;
Psychology, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Introduction: For humans, damage to the prefrontal lobe causes some of the most long-lasting deficits in cognition associated with traumatic brain injury (TBI). These impairments span many modalities due to the interconnected nature of the frontal cortex, ranging from memory impairments to attentional problems to poor decision-making and more. However, these types of behaviors have not been widely assessed following TBI in the rat, one of the most common animals used for models of brain injury. Methods: In the current study, we trained rats on the five-choice serial reaction time task. This task allows for the simultaneous assessment of attention (via accuracy), motivation (via omitted responses) and motor impulsivity (via premature responses) as well as a variety of latency measures. Rats were then subjected to one of four injury severities: sham (no injury), mild-TBI, moderate-TBI or severe-TBI and re-assessed on the task. Results: We found that TBI caused impairments in a severity-dependent fashion across all measures. Severe-TBI animals showed the largest scale deficits in attention,

motivation, motor impulsivity and response latency and most showed no evidence of recovery. Moderate- and Mild-TBI animals demonstrated deficits across these measures, but also some recovery over a 20 day period. Conclusions: Complex tasks such as the one used in this study represent a novel way of assessing TBI in animals. By modeling deficits that are very directly relatable to the human condition, we can improve our animal models of brain injury considerably. These can then be used to assess therapeutics in a meaningful fashion.

Disclosures: C. Vonder Haar: None. **C.A. Winstanley:** None.

Nanosymposium

119. Corticolimbic Circuits and Decision-Making

Location: 206

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 119.04

Topic: F.02. Animal Cognition and Behavior

Support: NSERC

Title: Spatiotemporal distribution of ventral striatal local field potentials revealed by high-density silicon probes

Authors: *J. E. CARMICHAEL, M. A. A. VAN DER MEER;
Biol., Univ. of Waterloo, Waterloo, ON, Canada

Abstract: The ventral striatum (vStr) is an anatomically and functionally heterogenous structure, yet its local field potential displays remarkably homogeneous oscillations across several frequency bands, including delta (~4Hz), theta (6-10Hz), beta (13-20Hz), low-gamma (40-60Hz) and high-gamma (70-100Hz). The spiking activity of many vStr neurons, including putative fast-spiking interneurons and medium spiny projection neurons, is systematically related to one or more of these rhythms, demonstrating that vStr LFP oscillations are locally relevant. Thus, it is possible that vStr LFPs play a role in the dynamic routing of information through selective coherence with different in- and output areas, and/or that vStr LFPs may serve to organize local cell assemblies. However, it is currently unclear what components of vStr LFP oscillations are locally generated de novo, inherited from one or more already oscillating inputs, and volume-conducted from nearby structures. To obtain a more detailed view of vStr LFP oscillations, we chronically implanted regularly spaced, 64-channel electrode arrays into the rat vStr, and recorded spike and LFP data both off-task and during a reinforcement learning task. These arrays sampled either a 1.4 x 0.2 mm or a 1.4 x 1.4 mm area across the core and shell areas of the vStr.

In multiple rats (n = 4), these arrays consistently recorded non-uniform distributions of LFP power across the vStr, which could not be accounted for by variations in impedance values pre- or post-surgery. These distributions of LFP power showed remarkable overlap across frequency bands including low- and high-gamma. Furthermore, analysis of gamma phases showed instances of gradual phase gradients across sites, which similarly overlapped between frequency bands. Taken together, these results suggest that different LFP components are not associated with specific vStr subregions, but have a common source in the vStr rather than resulting from volume conductance. Future work can build on these observations to evaluate further the hypothesis that vStr LFP oscillations play a functional role in information processing.

Disclosures: J.E. Carmichael: None. **M.A.A. van der Meer:** None.

Nanosymposium

119. Corticolimbic Circuits and Decision-Making

Location: 206

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 119.05

Topic: F.02. Animal Cognition and Behavior

Support: CIHR Doctoral Research Award

Title: Prefrontal cortical inactivations decrease willingness to expend cognitive effort on a rodent cost/benefit decision-making task

Authors: *C. A. WINSTANLEY[1], P. J. COCKER[2], J. G. HOSKING[2];
[1]Univ. British Columbia, Vancouver, BC, Canada; [2]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Personal success often necessitates expending greater effort for greater reward but, equally important, also requires judicious use of our limited cognitive resources (e.g. attention). Deficits in such effortful decision making accompany a number of mental illnesses including depression, schizophrenia, and attention-deficit/hyperactivity disorder. Animal models have implicated brain regions such as the basolateral amygdala and anterior cingulate cortex in effort-based decision making; on the other hand, human studies identify areas deemed inessential in animal paradigms (e.g. medial and lateral prefrontal cortex). However, choices in animal models typically vary in their physical requirements, such as scaling a barrier or pressing a lever more times for greater rewards, whereas choices in human studies traditionally vary in their cognitive requirements. Here we utilize a rat Cognitive Effort Task (rCET) to probe the prefrontal cortex's

contribution to effortful decision making. In the rCET, animals can choose either an easy trial, where the visuospatial attentional demand is low but the potential reward (sugar) is small, or a difficult trial on which both the attentional demand and available reward are greater. Temporary inactivation of the infralimbic (IL) and prelimbic (PrL) cortices showed both overlapping and dissociable effects: both IL and PrL inactivations decreased all animals' willingness to expend mental effort; IL inactivations also increased measures of motor impulsivity, whereas PrL inactivations greatly impaired animals' ability to perform the task. These data imply that the prefrontal cortex contributes to an attentional resource pool, and when these resources are diminished, animals will shift their choice (via other brain regions) toward a more judicious strategy. Thus, this study suggests one novel therapeutic approach to deficits in effort expenditure may be to focus on the resources that such decision making requires, rather than the decision-making process per se.

Disclosures: J.G. Hosking: None. **P.J. Cocker:** None. **C.A. Winstanley:** None.

Nanosymposium

119. Corticolimbic Circuits and Decision-Making

Location: 206

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 119.06

Topic: F.02. Animal Cognition and Behavior

Support: Fondation FYSSEN

Title: Cortico-striatal and striato-cortical flow of information are associated with high and low gamma oscillations respectively

Authors: *J. CATANESE, M. A. A. VAN DER MEER;
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Abstract: The ventral striatum (vStr) is an area of anatomical convergence, where inputs from cortical areas, hippocampus, amygdala, and brainstem nuclei interact at the single neuron and local circuit level. Appropriate integration and switching between these inputs is thought to be key to vStr's contribution to motivated behavior; however, how this is accomplished remains unclear. Recent work has revealed a rich palette of oscillations in ventral striatal local field potentials (LFPs), including distinct low-gamma (45-55Hz) and high-gamma (70-90Hz) events, which are systematically related to behavior and spiking activity. These observations raise the possibility that gamma oscillations may play a role in controlling the flow of information through

the vStr. As a first step in evaluating this hypothesis, we recorded LFPs from vStr and its medial prefrontal cortex (mPFC) input as rats foraged for rewards on a linear track. We found clear instances of coherent gamma events between vStr and mPFC during the reward expectation and delivery epochs, as well as during rest. Interestingly, high-gamma events in mPFC systematically led those in vStr, whereas in contrast, low-gamma events in mPFC systematically lagged those in vStr. This effect was robust across animals (n = 4) and multiple directional measures, including cross-correlations of instantaneous gamma power, and phase slope indices (PSI). These results suggest that high-gamma may reflect information flow from mPFC to vStr, whereas low-gamma indicates information flow from vStr indirectly to mPFC. Consistent with this idea, vStr high-gamma events tended to be followed by low-gamma events but not vice versa. Finally, low and high gamma events were associated with distinct ensemble spiking patterns, as demonstrated by a classifier which reliably predicted the identity of the LFP event based on spike counts of simultaneously recorded neurons. Taken together, these results indicate that vStr gamma oscillations behave in a manner consistent with dynamic routing of information through cortico-striatal loops, and enable further experiments that elucidate the role of these oscillations in coordinating multiple converging inputs.

Disclosures: J. Catanese: None. **M.A.A. van der Meer:** None.

Nanosymposium

119. Corticolimbic Circuits and Decision-Making

Location: 206

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 119.07

Topic: F.02. Animal Cognition and Behavior

Support: Children's Healthcare of Atlanta

NIH P51OD11132

NIH DA034808

NIH P30NS055077

NIH DA015040

Title: Rho-kinase inhibition augments goal-directed decision-making and blocks habitual responding for cocaine

Authors: *A. M. SWANSON[1,2], L. M. DEPOY[1,2], S. L. GOURLEY[1,2];
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Abstract: Considerable evidence indicates that both humans and rodents can learn to associate specific actions with their outcomes. With repeated performance, drugs, or stressor exposure, these actions can assume stimulus-elicited, or 'habitual,' qualities that are resistant to change. A growing literature has identified mechanisms by which decision-making strategies shift from action-outcome-based to stimulus-response-based habit systems, but *reversing* habits has proven difficult. Here, we isolated the role of Rho-kinase (ROCKII), a key regulator of the actin cytoskeleton, within the prelimbic cortex. First, we show that deep-layer prelimbic cortical dendritic spine density *predicts decision-making strategies*, such that higher densities are associated with stimulus-response habits, while lower densities are associated with engagement in action-outcome response strategies, as determined by behavioral sensitivity to outcome devaluation and response-outcome contingency degradation. Next, we show that fasudil, a Rho-kinase inhibitor, transiently reduces prelimbic cortical dendritic spine density and restores goal-directed decision-making in mice that have otherwise developed stimulus-response habits due to extensive response training. Our findings further suggest that fasudil acts on the consolidation of new response-outcome conditioning. Finally, pairing fasudil with the devaluation of a cocaine reinforcer results in a marked delay in the acquisition of a new response for intravenous cocaine delivery. Together, these findings suggest that Rho-kinase inhibition promotes action-outcome decision-making by augmenting the plasticity of deep-layer medial prefrontal cortical dendritic spines, and that it has therapeutic potential in the context of cocaine abuse.

Disclosures: A.M. Swanson: None. **L.M. DePoy:** None. **S.L. Gourley:** None.

Nanosymposium

119. Corticolimbic Circuits and Decision-Making

Location: 206

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 119.08

Topic: F.02. Animal Cognition and Behavior

Support: NSERC Discovery Grant 402642

Title: The role of the nucleus accumbens core in approach avoidance conflict resolution

Authors: *L. M. HAMEL[1], R. ITO[2];
[1]Univ. of Toronto Scarborough, Toronto, ON, Canada; [2]Univ. of Toronto, Toronto, ON, Canada

Abstract: The most fundamental decisions that an organism must make in order to ensure survival and reproduction involve the resolution of approach versus avoidance directed toward environmental signals. In mammals, regions in the cortico-limbic-striatal have been implicated in the processing of motivationally significant information. The nucleus accumbens (NAc), in particular, can be thought to co-ordinate the control over motivated behaviour by environmental cues, taking account of homeostatic, affective states, and behavioural contexts. There is also some evidence indicating that control over appetitively and aversively motivated behavior is anatomically and functionally segregated at the level of the NAc, with a mapping of expression of appetitive vs. aversive behaviours along a rostral-caudal gradient of the NAc shell. It remains to be determined whether the core region of the NAc demonstrates a topographical gradient in the control over approach vs. avoidance behaviours. The current study therefore examined its role of the NAc core in mediating the resolution of approach versus avoidance, using a novel behavioural paradigm requiring a decision in the presence of cues which simultaneously predict both appetitive and aversive outcomes. Male Long-Evans rats were trained via sequential exploration of a maze to associate visual cues (panels having different colour, texture, and reflective properties) with different outcomes (sucrose reward, shock, or no consequences). After 8 conditioning sessions, rats underwent a conditioned cue preference/avoidance test to determine whether the cued contingencies had been learned. Rats then received an intracerebral microinfusion of the GABA agonists baclofen and muscimol, or saline vehicle to disrupt local activity in the NAc. The rats were then tested in two arms of the radial maze, with one arm containing both positively and negatively valenced cues, and a second arm containing the neutral cue. The time spent exploring both arms was measured. Preliminary data demonstrate that animals undergoing disruption of the caudal NAc core spent a lower proportion of time in the conflict arm as compared with neutral arm, and as compared with vehicle animals. The results indicate a motivational bias in the direction of aversion and suggest that this region may be important in arbitrating between approach and avoidance. Considerations of sub-regional topography and functional connectivity will be discussed.

Disclosures: **L.M. Hamel:** None. **R. Ito:** None.

Nanosymposium

119. Corticolimbic Circuits and Decision-Making

Location: 206

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 119.09

Topic: F.02. Animal Cognition and Behavior

Support: HHMI

Affymetrix Bio-X Fellowship

Stanford MSTP

Title: Population encoding of value and choice signals as separable, dynamic neural ensembles in macaque orbitofrontal cortex

Authors: *D. L. KIMMEL[1], A. RANGEL[2], W. T. NEWSOME, III[3];
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Abstract: Orbitofrontal cortex (OFC) has long been implicated in value-based decision-making. However, the signals observed in OFC are complex, with individual neurons representing multiple task-relevant signals, such as stimulus value, behavioral choice, and expected reward. It was therefore unclear how the mixed responses of a given neuron might contribute to the distinct cognitive-behavioral functions theoretically subserved by these various signals. We recorded from macaque OFC while monkeys performed a cost-benefit decision-making task that required the animal to evaluate an offer and then maintain an effortful response so as to earn the promised reward (1). We found that the animal was sensitive to the balance of cost and benefit. That is, its willingness to accept an offer increased monotonically as we increased the benefit while keeping the cost constant. Within OFC, we confirmed that representations of value, choice, and expected reward were mixed at the level of single neurons. However, by examining the population response across hundreds of serially recorded neurons (2), we found that separable patterns of neural activity represented each of these task-relevant variables. Moreover, each pattern represented a task-relevant signal stably over a discrete temporal epoch that aligned with behaviorally relevant events. For instance, an early pattern represented the offer value briefly during presentation of the offer, but a different pattern represented expected reward during the period of sustained effort. Similarly, one pattern of activity represented the animal's choice prior to the outcome of the trial, but after the trial ended, this choice representation abruptly transitioned to a different pattern of activity that carried the now-previous choice information into the next trial. Taken together, we offer a novel approach to understanding value and choice signals within OFC, which are mixed at the level of single neurons but are carried by separable patterns of neural activity at the level of the population. The separability and temporal dynamics of these patterns suggest they may subserve distinct cognitive-behavioral functions essential for cost-benefit decision-making. 1. D. L. Kimmel, A. Rangel, & W. T. Newsome. (2010) "Cost-benefit decisions and value representations in the primate orbitofrontal cortex." *Society for Neuroscience Annual Meeting*, San Diego, CA, November 15, 2010. 2. V. Mante, D. Sussillo, K. V. Shenoy, & W. T. Newsome (2013) "Context-dependent computation by recurrent dynamics in prefrontal cortex." *Nature*, 503: 78-84.

Disclosures: D.L. Kimmel: None. **A. Rangel:** None. **W.T. Newsome:** None.

Nanosymposium

119. Corticolimbic Circuits and Decision-Making

Location: 206

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 119.10

Topic: F.02. Animal Cognition and Behavior

Support: Wellcome Trust 091593/Z/10/Z

Title: Parsing the role of the hippocampus in avoidance and exploration

Authors: *E. LOH[1,2], M. GUITART-MASIP[3], D. BACH[4], E. DUZEL[5], R. J. DOLAN[2]; [1]Univ. Col. London, London, United Kingdom; [2]Wellcome Trust Ctr. for Neuroimaging, London, United Kingdom; [3]Karolinska Institutet, Stockholm, Sweden; [4]Zurich Univ. Hosp. for Psychiatry, Zurich, Switzerland; [5]Inst. of Cognitive Neurosci., London, United Kingdom

Abstract: While evidence from the human and animal literature points towards a prominent role for the hippocampus in the anxiety, avoidance and exploration, previous studies have not been able to map hippocampal contributions to more specific component processes (such as the tracking of aversive conditions, the generation of exploratory behaviours, or the generation of behavioural inhibition). To examine this issue in detail, we combined fMRI with a novel, non-spatial decision-making paradigm that invoked conflict between approach and avoidance. Subjects evaluated gambles that probabilistically indicated gain or loss, and decided whether to accept or reject them. Subjects were also given the option to 'explore' before deciding to accept or reject each gamble - doing so would reveal, for a fee, a hint that reduced outcome uncertainty on that particular trial. By separating the options of 'rejecting' gambles and 'exploring' them (i.e. performing risk assessment), we were thus able to parse any potential hippocampal involvement more finely than in previous experiments on behavioural inhibition and anxiety. The bilateral anterior hippocampus alone showed a greater response to gambles that subjects chose to reject, and activation of these regions was additionally correlated with state and trait anxiety scores of personality across all subjects. In contrast, the decision to explore a gamble (i.e. perform risk assessment) was associated with activation of a network of fronto-striatal regions, as well as with decreased activation of additional clusters in the anterior hippocampus. Our findings suggest that the hippocampus plays an active role in the decision to avoid threats, rather than in the passive tracking of aversive conditions. They further suggest that, in circumstances of approach-avoidance conflict, hippocampal contributions may relate more closely to behavioural inhibition and avoidance, rather than to the generation of exploratory 'risk-assessment' behaviours.

Disclosures: **E. Loh:** None. **M. Guitart-Masip:** None. **D. Bach:** None. **E. Duzel:** None. **R.J. Dolan:** None.

Nanosymposium

119. Corticolimbic Circuits and Decision-Making

Location: 206

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 119.11

Topic: F.02. Animal Cognition and Behavior

Support: R01 MH084911

R01 MH093665

Title: Primary visual cortex engenders timed actions

Authors: ***V. K. NAMBOODIRI**[1], M. HUERTAS[2], K. J. MONK[1], H. Z. SHOUVAL[2], M. G. HUSSAIN SHULER[1];
[1]Johns Hopkins Univ., BALTIMORE, MD; [2]Univ. of Texas, Houston, TX

Abstract: The production of a behavior often requires animals to sense the external world, make decisions based on that information and generate appropriate motor responses. The canonical view of brain organization is that these functions are performed hierarchically by sensory, association, and motor areas respectively. The role of sensory areas_especially primary sensory areas_has long been regarded as providing a faithful representation of the external world. However, this view has recently been challenged by observations that sensory cortices represent not only stimulus features but also non-sensory information. In the visual modality, it has been shown that V1 can predict learned intervals between a stimulus and a reward and that the ability to learn such intervals depends on cholinergic input from the basal forebrain. In fact, such timing responses can be trained even within an isolated in-vitro preparation of V1, demonstrating that the site of learning is local to V1. However, whether such predictive signals in primary sensory areas can directly instruct and govern behavior is unclear. Here, we developed a novel visually-cued interval timing task to address this question. In this task, rats had to decide when to lick on a water spout following the delivery of a visual stimulus: the longer the animals waited (up to a target interval), the more water they received. Waiting longer than the target interval resulted in no water reward. The design of our current task was motivated to address whether V1 activity that reflects the average delay between stimulus and reward could be used to directly instruct the lapse of a target interval in order to time an action. We observed that well-trained animals in this

task wait a stereotyped interval (close to the target) after the stimulus before deciding to lick. Interestingly, single unit recordings showed responses that correlate with the timed action on a trial-by-trial basis. Specifically, we found neurons that convey the passage of time from the cue to the target interval as well as others that report the expiration of the target interval, potentially informing the timing of the behavioral response. Optogenetic perturbation of activity in V1 during the timed interval (but after cue offset) shifted timing behavior. Our results indicate that post-stimulus activity in V1 embodies the wait interval and governs the timing of the behavioral response. We show that a local recurrent network model of spiking neurons can reproduce our observations since single unit activity within this network showed trial-by-trial correlations with the action.

Disclosures: V.K. Namboodiri: None. **M.G. Hussain Shuler:** None. **K.J. Monk:** None. **M. Huertas:** None. **H.Z. Shouval:** None.

Nanosymposium

119. Corticolimbic Circuits and Decision-Making

Location: 206

Time: Sunday, November 16, 2014, 8:00 AM - 11:00 AM

Presentation Number: 119.12

Topic: F.02. Animal Cognition and Behavior

Support: R01-MH098899

F31-MH093099

Soros Fellowship

UPenn MSTP

Title: Dynamic encoding of errors by the anterior and posterior cingulate cortex in a changing world

Authors: *Y. LI[1], M. R. NASSAR[2], J. I. GOLD[1];
[1]Dept. of Neurosci., Univ. of Pennsylvania, Philadelphia, PA; [2]Brown Univ., Providence, RI

Abstract: Inferring the latent state of a changing world based on observable outcomes is a challenging task. Unexpected outcomes can arise from both random fluctuations in outcomes generated by an otherwise stable process (noise) and changes in the underlying state of the world (changepoints). The degree to which a subject learns from an outcome should therefore be

adaptive. For example, learning should be greatest in response to errors in a low-noise environment (corresponding to a high “changepoint probability”), and just after such changepoints, when new information is most informative (corresponding to a high “estimation uncertainty”). To identify how and where in the brain these aspects of adaptive learning are encoded, we recorded from individual neurons in both the anterior (ACC) and posterior cingulate cortex (PCC) of two monkeys performing a ten-alternative saccadic-choice task. This task included both static fluctuations (noise) as well as abrupt changes (changepoints) in the identity of the rewarded target. The monkeys’ behavior showed key signatures of adaptive learning, including particularly high sensitivity to errors (i.e., a tendency to switch their choice of target) when noise was low and when changepoints had recently occurred. Approximately half of the units in both ACC (53 out of 99 recorded units; $P<0.05$, t-test) and PCC (79/161; $P<0.05$, t-test) differentiated between correct and error trials within 500 ms of visual feedback indicating the identity of the reward target. In a subset of the ACC units (15/99; $P<0.05$, F-test), this error encoding depended on the number of trials since the last changepoint. By contrast, in the PCC, error encoding depended primarily on the magnitude of the error, scaled by the noise (41/161; $P<0.05$, F-test). These results suggest that the encoding of errors in the ACC and PCC map onto the computationally distinct quantities of estimation uncertainty and changepoint probability, respectively. These brain areas thus may make complementary contributions to the rational adjustment of learning in a changing world.

Disclosures: Y. Li: None. **M.R. Nassar:** None. **J.I. Gold:** None.

Nanosymposium

197. Molecular and Functional Biomarkers of Neurodegeneration

Location: 144A

Time: Sunday, November 16, 2014, 1:00 PM - 2:45 PM

Presentation Number: 197.01

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

Support: CCCRP H_003_2014_WRAIR

Title: Long-term changes in cleavage processing of Alzheimer’s disease related factors APP and Tau following penetrating TBI

Authors: *C. M. CARTAGENA, A. MOUNTNEY, Z. RAMMELKAMP, A. SWIERCZ, D. A. SHEAR, F. C. TORTELLA, K. E. SCHMID;
Brain Trauma Neuroprotection and Neurorestoration, Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract: Traumatic brain injury (TBI) has been established as a risk factor the later development of Alzheimer's disease (AD). Historically both clinical and animal research investigating links between TBI and Alzheimer's disease exclude cases or models of penetrating brain injury, leaving a significant gap in knowledge. In addition, AD studies are most often conducted using familial mutations of amyloid precursor protein (APP) or Tau, mutations that may not represent disease progression in sporadic AD or TBI induced cleavage processing of wild type proteins. Here we investigate in rats the effects of penetrating non-ballistic (NB) brain injury and ballistic-like brain injury (PBBI) on APP and Tau cleavage processing in the acute (first week) and subacute (1 month) periods post-injury. PBBI was induced by probe insertion and the rapid inflation of a balloon injuring 10% of total brain volume while NB injury was induced with probe insertion alone. All changes were compared to sham control. Full length (FL) APP was unaltered with NB but decreased significantly with PBBI at 3 (68%) and7 days (47%). APP beta c-terminal fragments (βCTFs) were increased dramatically at 4 and 24 hr post PBBI (856% and 2942% respectively). Later increases (3 days, 193%; 7 day 729%) were more moderate but persistent, indicating a second wave of APP beta cleavage occurs with PBBI. NB induced a temporally similar but reduced amplitude pattern of increases that was only significant at 7 days (547%). Full length Tau was unaltered at 4 hr but decreased progressively with both NB and PBBI starting at 24 hr and culminating in decreases of 87% and 96% respectively by 7 days post-injury. Importantly, a 22 kDa Tau fragment, known for its involvement in tauopathies, including Alzheimer's disease, increased dramatically following PBBI. Increases also showed a biphasic pattern with more substantial increases at 4hr (1541%) and 24 hr (2367%) and second phase increases at 3 (1541%) and 7 days 2424%). Again NB induced a temporally similar but reduced amplitude pattern of increases which were significant at 4 (544%), and 24 hr (893%) and 7 days (2313%). Pilot studies 1 month post PBBI indicate FL APP is unaltered and βCTFs trend up but are not significantly increased. However, FL Tau remains decreased (68%) and Tau 22 kDa fragment increases persist (468%). These studies show that penetrating brain injury dramatically alters neuropathologic factors related to AD into the subacute period post-injury, indicating that continued research of the relationship between penetrating brain injury and AD is warranted. Ongoing studies will determine if Tau pathology can be detected at more chronic time-points.

Disclosures: C.M. Cartagena: None. **A. Mountney:** None. **Z. Rammelkamp:** None. **A. Swiercz:** None. **D.A. Shear:** None. **F.C. Tortella:** None. **K.E. Schmid:** None.

Nanosymposium

197. Molecular and Functional Biomarkers of Neurodegeneration

Location: 144A

Time: Sunday, November 16, 2014, 1:00 PM - 2:45 PM

Presentation Number: 197.02

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

Support: 5P50AG005681-29

Title: Absolute quantitation of Apolipoprotein E isoforms in human cerebrospinal fluid and brain

Authors: *A. T. BAKER-NIGH[1], K. G. MAWUENYEGA[1], V. OVOD[1], H. ZAKERI[1], T. KASTEN[1], R. J. BATEMAN[1,2,3];
[1]Neurol., Washington Univ. Sch. of Med., Saint Louis, MO; [2]Charles F. and Joanne Knight Alzheimer's Dis. Res. Ctr., Saint Louis, MO; [3]Hope Ctr. for Neurolog. Disorders, Saint Louis, MO

Abstract: Risk for Alzheimer's Disease (AD) is correlated with Apolipoprotein-E (ApoE) genotype. In humans, the ApoE gene has three major allelic variants that differ by single cystine-arginine replacements. The prevalence for each allele is 78% ApoE3, 15% ApoE4, and 7% ApoE2. While ApoE3 is associated with baseline AD risk and ApoE2 confers decreased risk, ApoE4 is implicated in up to half of sporadic AD cases. The effect is dose-dependent, with ApoE4 heterozygotes 3-fold more likely and homozygotes 12-fold more likely to develop AD. ELISA-based measures of ApoE protein in the CNS do not consistently demonstrate an increase or decrease in ApoE4 levels in carriers. Liquid chromatography with selected reaction monitoring mass spectrometry (LC/SRM) enables the simultaneous detection and quantitation of ApoE isoforms. CSF and Brain Samples: CSF was obtained from 83 individuals (n=42 amyloid positive by CSF Amyloid-β 42/40 ratio or PiB-PET score, and 41 age-matched controls; 44 ApoE33, 35 ApoE34, and 4 ApoE44) and brain from 60 cognitively normal individuals with a range of ApoE genotypes. Estimating relative concentrations: Cross-titrated dilution curves of labeled ApoE3 (increasing) and ApoE4 (decreasing) heavy-arginine (13C6 15N4 L-arginine) media from immortalized murine astrocytes expressing human ApoE were spiked with a consistent volume of mixed unlabeled ApoE3/ApoE4 media to determine the ratio at which common peptides were detected equally. Similar cross-titration experiments were performed using equilibrated labeled media and pooled cerebrospinal fluid (CSF) from ApoE33 or ApoE44 homozygous cases. Consequently, separate and combined standard curves using E33 and E44 CSF were produced. Affinity purification and sample preparation: ApoE was isolated from CSF and brain spiked with media internal standard by affinity purification overnight using Liposorb, a lipophilic absorbant, or by immunoprecipitation using the WUE4 antibody. Samples were then denatured, reduced, and alkylated, followed by protein digestion with trypsin. ApoE isoform-specific peptides LGADMEDVCGR (E3) and LGADMEDVR (E4)and 4 common peptides were selected for analysis. Samples were then analyzed on a NanoAcquity LC coupled to a TSQ Vantage Mass Spectrometer for selected reaction monitoring (SRM) analysis. In this cohort, ApoE4 homozygote CSF demonstrated a decrease of up to 50% in ApoE amount, confirmed by

both specific and common peptide measures. ApoE4 was consistently higher (by ~11%) than ApoE3 in ApoE34 heterozygous cases, and similar in amount to ApoE44 homozygotes. ApoE33 and ApoE34 individuals had similar amounts of total ApoE in their CSF.

Disclosures: **A.T. Baker-Nigh:** None. **K.G. Mawuenyega:** None. **V. Ovod:** None. **H. Zakeri:** None. **T. Kasten:** None. **R.J. Bateman:** 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.; Merck, Research Collaboration, DIAN-TU, NIH U-01-AG042791, DIAN-TU, Alzheimer's Association, Zenith Fellows Award, Alzheimer's Association. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Avid Radiopharmaceuticals, DIAN-TU (donation of imaging supplies). D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents' (e.g., speakers' bureaus); Roche, Invited Speaker. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C2N, Co-Founder/Part Owner. F. Consulting Fees (e.g., advisory boards); Novartis, Sanofi, IMI.

Nanosymposium

197. Molecular and Functional Biomarkers of Neurodegeneration

Location: 144A

Time: Sunday, November 16, 2014, 1:00 PM - 2:45 PM

Presentation Number: 197.03

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

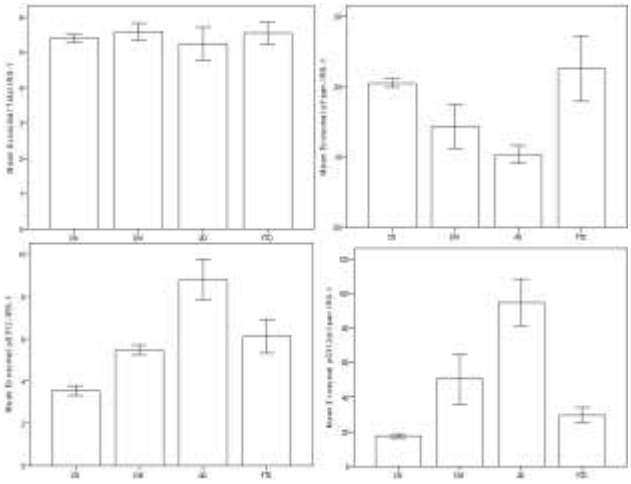
Support: Intramural Research Program of the NIA/NIH

Title: Neural origin plasma exosomes provide novel biomarkers for brain insulin resistance in Alzheimer's disease

Authors: ***D. KAPOGIANNIS**[1,2], A. BOXER[3], E. L. ABNER[5], A. BIRAGYN[1], U. MASHARANI[4], L. FRASSETTO[4], R. C. PETERSEN[6], B. L. MILLER[3], E. J. GOETZL[4]; [1]Natl. Inst. on Aging (NIA/NIH), Baltimore, MD; [2]Neurol., Johns Hopkins Univ., Baltimore, MD; [3]Neurol., [4]Med., UCSF, San Francisco, CA; [5]Univ. of Kentucky, Lexington, KY; [6]Neurol., Mayo Clin., Rochester, MN

Abstract: Introduction: Brain insulin resistance (IR) occurs in Alzheimer's disease (AD), even without peripheral IR. Brain IR is pathogenically important and the target of clinical trials (intranasal insulin, exenatide), but no biomarker of brain IR exists. Recently, high Ser- and low

Tyr-phosphorylated IRS-1 (insulin receptor substrate-1) were proposed as ex-vivo markers of brain IR. Exosomes are endosome-derived vesicles released by various cells (including neurons) and contain proteins reflecting their cellular source. We hypothesized that neural-origin exosomes can be derived from plasma and provide brain IR biomarkers. Methods: Cross-sectional: 48 patients with AD without diabetes, 20 elderly cognitively normal (CN) subjects with diabetes, 16 patients with Frontotemporal Dementia (FTD), and 84 CN controls. Longitudinal: 22 patients with AD with samples 1-10 years before diagnosis. We isolated exosomes from plasma and derived a portion enriched for neural origin by means of expressing NCAM/ L1-CAM. We quantified total IRS-1, p-Ser312-IRS-1, p-panY-IRS-1 (Tyr phosphorylated form) in neural-origin-enriched exosomes and calculated p-Ser312/p-panY ratios. We examined their performance in diagnostic classification with Discriminant Classification (cross-validated by leave-1-out) and Receiver Operating Characteristic (ROC) analyses. Results: AD patients had several-fold higher p-Ser312-IRS-1 and Ser312/p-panY ratios and lower p-panY-IRS-1 than CN, diabetes, and FTD controls (Figure displays means and 95% C.I.; p 98% of AD patients vs. CN controls. The Ser312/p-panY ratio achieved a 0.99 ROC area under the curve. Longitudinally, preclinical and clinical p-Ser312-IRS-1, p-panY-IRS-1, and Ser312/p-panY were indistinguishable; preclinical levels of all three differed vs. controls ($p<0.001$). Conclusions: We propose p-Ser312-IRS-1, p-panY-IRS-1, and Ser312/p-panY from neural-origin plasma exosomes as biomarkers of brain IR in AD. These markers near-perfectly discriminate AD patients vs. CN, diabetes and FTD controls and may predict future AD diagnosis.



Disclosures: D. Kapogiannis: None. **A. Boxer:** None. **E.L. Abner:** None. **A. Biragyn:** None. **U. Masharani:** None. **L. Frassetto:** None. **R.C. Petersen:** None. **B.L. Miller:** None. **E.J. Goetzl:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Nanosomix, Inc..

Nanosymposium

197. Molecular and Functional Biomarkers of Neurodegeneration

Location: 144A

Time: Sunday, November 16, 2014, 1:00 PM - 2:45 PM

Presentation Number: 197.04

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

Support: APVV-0088-10

VEGA 2/0036/11

Brain Centre of SAS

Title: Stress-induced changes in tau proteins and catecholamines in a rat model of Alzheimer´s neurodegeneration

Authors: *R. KVETNANSKY[1], K. LEJAVOVA[2], P. NOVAK[3], A. OPATTOVA[3], K. ONDICOVA[2], L. HORVATHOVA[2], P. VARGOVIC[2], G. MANZ[4], B. MRAVEC[2], P. FILIPCIK[3], M. NOVAK[3];
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Abstract: Stress is one of the factors suspected of promoting neurofibrillary degeneration in Alzheimer´s disease (AD). The aim of this study was to investigate the mutual influences between stress, brain catecholamines (CA) and pathological post-translational modifications of tau protein. The influence of stress on progression of neurodegeneration has been investigated using rats over-expressing human truncated tau protein. Furthermore, corticotropin-releasing hormone (CRH)-knockout mice were utilized to elucidate the role of CRH and corticosteroids in an impact of stress on tau protein modifications. A total of 14 brain areas were analyzed for levels of hyperphosphorylated tau protein, CA, and gene expression of CA-biosynthetic enzyme - tyrosine hydroxylase (TH) in control, singly, and repeatedly stressed animals by immobilization for 2 hours daily (IMO). In both experimental models we found significant hyperphosphorylation of several AD-associated epitopes on tau proteins (pT181, pS202/T205, Ser396/Ser404). Tauopathy induced altered norepinephrine levels in many investigated brain areas and increased expression of TH in brainstem areas, e.g. in the locus coeruleus (A6 area), in A1, A5 areas, etc. The HPA axis has been found to be an important mediator of the hyperphosphorylation response of tau proteins to stress. We have shown that stress induces tau protein phosphorylation throughout the brain, and that absence of CRH delays the onset of hyperphosphorylation. In chronic stress, CRH-producing animals showed an attenuation of the stress response, while

CRH-knockout mice displayed an exaggerated phosphorylation response. This indicates a more complex role of CRH in tau phosphorylation than the current state of the art shows. Our results suggest that stress-induced pathological phosphorylation of tau proteins represents one of the potential mechanisms, which can lead to misfolding of tau proteins and thus to acceleration of neurodegeneration. The results suggest a close interaction between neurofibrillary degeneration and stress, especially repeated or chronic.

Disclosures: **R. Kvetnansky:** None. **K. Lejavova:** None. **P. Novak:** None. **A. Opattova:** None. **K. Ondicova:** None. **L. Horvathova:** None. **P. Vargovic:** None. **G. Manz:** None. **B. Mravec:** None. **P. Filipcik:** None. **M. Novak:** None.

Nanosymposium

197. Molecular and Functional Biomarkers of Neurodegeneration

Location: 144A

Time: Sunday, November 16, 2014, 1:00 PM - 2:45 PM

Presentation Number: 197.05

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

Support: Center for Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB), Göttingen, Germany

Title: Super-resolution microscopy of cerebrospinal fluid biomarkers: a novel tool for diagnostic research in Alzheimer's Disease

Authors: ***W. I. ZHANG**[1,2], G. ANTONIOS[3], A. RABANO[4], T. PENA-CENTENO[5], T. A. BAYER[3], S. BONN[5], A. SCHNEIDER[6,2,5], S. O. RIZZOLI[1,2];
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Abstract: Beta-amyloid (Abeta) and tau oligomerization play a critical role in Alzheimer's Disease (AD) pathology. It is currently thought that they should serve as diagnostic markers for AD. Ideally, the perfect diagnostic tool would not only quantify the porportion of Abeta and tau monomers and different aggregate species, but also the sizes of the aggregates. This has not been possible to date since the assemblies are smaller than the diffraction limit of fluorescence

microscopy (~200 nm). To address this, we turned to stimulated-emission depletion (STED) microscopy. This technique has a high enough precision to differentiate dimers/trimers of Aβ from larger aggregates produced in vitro. We immunostained cerebrospinal fluid (CSF) of 37 AD patients and 23 controls and measured the number of Abeta and tau aggregates, as well as their sizes and intensities. In preliminary analysis, a linear discriminant used these parameters to achieve an accuracy of ~97.0% in discriminating AD patients from controls. In contrast, a similar analysis based only on Abeta and tau concentrations, measured via ELISA, resulted in a precision of ~89.7%. In conclusion, we introduce here a precise diagnostic method for AD which may also be used to predict AD at pre-symptomatic stage. In addition, this technique constitutes an unprecedented application of super-resolution microscopy to the medical diagnostic field.

Disclosures: W.I. Zhang: None. **G. Antonios:** None. **A. Rabano:** None. **T. Pena-Centeno:** None. **T.A. Bayer:** None. **S. Bonn:** None. **A. Schneider:** None. **S.O. Rizzoli:** None.

Nanosymposium

197. Molecular and Functional Biomarkers of Neurodegeneration

Location: 144A

Time: Sunday, November 16, 2014, 1:00 PM - 2:45 PM

Presentation Number: 197.06

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

Support: National Science Foundation

NIH: AG017586

NIH: AG032953

NIH: AG038490

NIH: AG043503

NIH: NS044266

NIH: NS053488

Title: Neural correlates of verbal memory and lexical retrieval in Logopenic Variant of Primary Progressive Aphasia

Authors: *K. WIN[1,2], J. PLUTA[3], P. YUSHKEVICH[3], D. WOLK[2], M. GROSSMAN[1,2]; [1]Neurol. Dept, Penn Frontotemporal Degeneration Ctr., Philadelphia, PA; [2]Neurosci. Grad. Group, [3]Penn Image Computing and Sci. Lab., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Motivation: Logopenic variant of primary progressive aphasia (lvPPA) is characterized by poor repetition of phrases and sentences, and limited word-finding. Recently, Flanagan et al. 2014 showed verbal memory deficit in lvPPA but the basis for this is unclear. One possibility is related to discrete atrophy in medial temporal lobe (MTL) substructures, including Cornu Ammonis (CA1), dentate gyrus (DG), subiculum (SUB), entorhinal cortex (ERC), Brodmann areas (BA) 35 and 36. Much research has shown posterior perisylvian atrophy but no MTL atrophy. lvPPA is often associated with Alzheimer's disease (AD), associated with MTL atrophy. Even if there is not overall MTL atrophy in lvPPA, MTL substructures might be differentially affected. T1 MRI of whole-brain grey matter (GM) may be insensitive to detect atrophy of MTL substructures associated with verbal episodic memory (EM) deficit. A second possibility is impaired lexical retrieval may interfere with EM recall. A more reliable assessment of EM may require a recognition testing. Here, we related verbal EM recall and recognition as well as lexical retrieval in lvPPA to MTL substructures using a specialized high resolution T2 MRI sequence, and to GM atrophy using T1 MRI. Methods: Both lvPPA (n=11) and elderly controls (Ctl, n=22) were matched in age, sex, education, and intracranial volume. All subjects underwent T1 MRI as well as a T2 MRI, which maximizes visualization of the dark band separating CA from DG. A multi atlas algorithm was applied to automatically label CA1, DG, SUB, ERC, BA35 and BA36. We used Philadelphia Verbal Learning Test (PVLT) to assess verbal episodic memory and Boston Naming Test (BNT) to measure lexical retrieval. Regression analyses were performed. Results: Compared to Ctl, lvPPA patients performed poorly on forward digit span ($p<0.001$), BNT ($p<0.025$), and PVLT delayed recall ($p<0.008$) although their recognition memory was intact ($p>0.5$). Significant atrophy of MTL substructures was found in right BA35, bilateral CA1 and SUB in lvPPA. Regression analyses showed that only PVLT recall, but not BNT, is associated with left CA1 ($r=0.69$, $p=0.019$). Significant GM atrophy was found in temporal-parietal areas, including middle and inferior temporal, and superior parietal gryi: BNT deficit is related to left superior-parietal atrophy and PVLT to left posterior-inferior temporal atrophy, areas involved in lexical-retrieval and depth of processing effect on encoding of words. Conclusion: We found that specific MTL substructures and temporal-parietal areas are both affected in lvPPA, and regression analyses suggest that impaired lexical retrieval contributes significantly to EM deficits in lvPPA.

Disclosures: K. Win: None. **J. Pluta:** None. **P. Yushkevich:** None. **D. Wolk:** None. **M. Grossman:** None.

Nanosymposium

197. Molecular and Functional Biomarkers of Neurodegeneration

Location: 144A

Time: Sunday, November 16, 2014, 1:00 PM - 2:45 PM

Presentation Number: 197.07

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

Title: Role of entorhinal cortex - hippocampal circuit in Alzheimer's disease mouse model

Authors: *S. A. HUSSAINI, K. DUFF;
Pathology and Cell Biol., Columbia Univ. Med. Ctr., New York, NY

Abstract: Spatial disorientation and confusion in familiar surroundings is an early symptom commonly seen in patients with Alzheimer's disease (AD). The brain regions, entorhinal cortex and hippocampus (known to be important for memory of space), are known to be one of the first regions to undergo pathological changes in Alzheimer's disease. By employing electrophysiological techniques in an AD mouse model we aim to understand the mechanism by which AD affects the neurons involved in spatial memory. Multiple electrodes were implanted in the CA1 region of the hippocampus and medial entorhinal cortex. Animals were allowed to explore an open field environment and subjected to a spatial task. While animals performed the spatial task the activity of neurons in entorhinal cortex and hippocampal was recorded. The hippocampal neurons-place cells- fire at specific locations in an environment representing animal's position. The neurons of entorhinal cortex-grid cells- fire in a grid-like pattern throughout the environment. We analyzed the properties of these neurons in AD mouse and compared with wild-type controls. The firing property of place cell of hippocampus and grid cell of entorhinal cortex was affected in AD mouse model mice. The neuronal properties correlated well with behavioral changes in the AD mice. Additionally, older cohorts performed poorly in behavioral tasks compared to younger cohorts and their neuronal properties were significantly altered. The neuronal properties of entorhinal cortex and hippocampus are significantly affected in an AD mouse model. These properties could potentially serve as a marker for detecting AD pathologies early in the disease.

Disclosures: S.A. Hussaini: None. **K. Duff:** None.

Nanosymposium

198. Neuroinflammation in Alzheimer's Disease

Location: 143A

Time: Sunday, November 16, 2014, 1:00 PM - 3:45 PM

Presentation Number: 198.01

Topic: C.05. Aging

Title: Oxidative stress blocks hippocampal LTP and it is rescue by cGMP, a link to AD

Authors: *L. BUITRAGO-SOTO[1,2], S. ANGULO[2], F. CARDOZO-PELAEZ[3], H. MORENO[2]; [1]Univ. Nacional De Colombia, Bogota, Colombia; [2]Pharmacol., SUNY Downstate Med. Ctr., Brooklyn, NY; [3]Biomed. and Pharmaceut. Sci., Univ. of Montana, Missoula, MT

Abstract: Recent data suggests that cupper levels have been found consistently elevated on MCI and AD and increased Cu levels can serve as predictor of conversion from MCI into AD. In our previous findings, oxidative environments induced by Copper and Ascorbic acid (Cu/Asc) led to the formation and elevation of oxo-8-GTP in a cell-free preparation and in cultured PC12 cells. It has been shown that oxo-8-GTP can act as an inhibitor of the Guanylate Cyclase (GC), significantly reducing cytoplasmatic levels of cGMP. LTP in the hippocampus depends on cGMP, and the inhibition of GC leads to block the induction of LTP in the CA3-CA1 synapsis. The goal of this study is to evaluate oxidative stress induced by Cu/Asc on synaptic plasticity and its molecular targets. Ventral horizontal hippocampal brain slices were obtained from 3 months old male mice. Slices were recorded using aCSF at 34°C. Stimulation electrode was placed in the Schaffer collaterals and evoked field Excitatory postsynaptic potential (fEPSPs) were recorded in the stratum radiatum of CA1. Slices were pre-incubated with Copper 10μM and Ascorbic Acid 1mM for 1h in order to induce the oxidative stress. Control slices were exposed to either normal aCSF or single bath application of copper or ascorbic acid. In a second set of experiments, the slices were pre-incubated with Cu/Asc, then washed-out with normal aCSF for 10 min (Cu/Asc/wo), and subsequently perfused with cGMP 100μM for 10 min. Stable baseline was recorded for 15 min, followed by induction of LTP with a high frequency stimulation (100Hz, 1 s). Slope was taken from the 10-90% of fEPSP, normalized to the baseline and used for the analysis. Synaptic stimulation was recorded for 1h after the induction the LTP and comparisons between groups were made 30 min after the induction. In the first series of the experiments, LTP was induced and maintained up to 1h in control slices. Slope was 2.9, 1.8 and 2 times higher than the baseline in slices exposed to aCSF, Cu and Asc respectively. Potentiation was significantly reduced in the slices exposed to Cu/Asc (1.4). In the second set of experiments, slices in the Cu/Asc/wo protocol had a significant reduction in the potentiation of LTP (1.6). But the potentiation was rescued with the bath application of cGMP (2.4). Thus, a mild oxidative stress challenge with Cu/Asc reduced the potentiation of LTP, which were reversed by the bath addition of cGMP. Our results, combined with previously published work with the Cu/Asc system provide a potential mechanism that can impact biochemical and electrophysiological components related to learning and memory and play a role in these neurological deficits.

Disclosures: **L. Buitrago-Soto:** None. **S. Angulo:** None. **F. Cardozo-Pelaez:** None. **H. Moreno:** None.

Nanosymposium

198. Neuroinflammation in Alzheimer's Disease

Location: 143A

Time: Sunday, November 16, 2014, 1:00 PM - 3:45 PM

Presentation Number: 198.02

Topic: B.11. Glial Mechanisms

Title: Microglial priming with interferon γ is essential for toll-like receptor 4-mediated neurotoxicity

Authors: *I. PAPAGEORGIOU[1], A. LEWEN[1], T. REGEN[2], U.-K. HANISCH[2], O. KANN[1]; [1]Med. Fac. Univ. of Heidelberg, Heidelberg, Germany; [2]Inst. of Neuropathology, Med. Ctr. of Georg-August-University, Göttingen, Germany

Abstract: Microglial activation has been associated with the pathogenesis of neurodegenerative diseases such as Parkinson´s disease, multiple sclerosis, Alzheimer´s disease and epilepsy with hippocampal sclerosis. Microglial cells not only remove dead/dysfunctional neurons, but are also proposed to directly induce neuronal death. However, mutual influences between innate (microglia) and adaptive immunity (lymphocytes, natural killer (NK) cells) might blur the individual roles of microglia in in vivo neurotoxicity. AIMS: We aimed to investigate microglial neurotoxicity in organotypic hippocampal slice cultures, a complex in situ approach free of adaptive immunity influences. Microglia are selectively targeted with the toll-like receptor 4 ligand, lipopolysaccharide (LPS), and the neurotoxic impact is investigated in presence or absence of the lymphocytic cytokine interferon γ (IFNγ). METHODS: We characterized microglial activation by a multi-dimensional approach combining high-order morphology, proinflammatory cytokine secretion profile (ELISA) and nitrite production (Griess). Cell numbers were estimated using unbiased, design-based stereology and single cells were reconstructed Neurolucida® for quantification of the soma and branching morphology. The neurotoxic impact was assessed by lactate dehydrogenase activity assay (LDH), neuronal (immuno)histochemistry (Nissl, parvalbumin and neurofilament staining) and extracellular electrophysiological recordings of spontaneous and evoked neuronal activity in the hippocampal CA3 subregion. RESULTS: 1) LPS did not induce neurotoxicity in the absence of interferon γ (IFNγ), despite activation-related morphological changes (i.e. process thickening and somatic enlargement), secretion of proinflammatory cytokines (TNFα, IL6) and production of nitrite.

Both LDH assay and detailed electrophysiology showed that LPS-induced activation was associated with only minor effects on neuronal excitability and short-term plasticity, but no neurodegeneration. 2) Co-incubation of LPS with IFNγ resulted in massive neurodegeneration associated with strong up-regulation of the microglial inducible nitric oxide synthase (iNOS) and high levels of nitrite in the supernatant. 3) LPS/IFNγ toxicity was reversible by pharmacological blockade of iNOS. CONCLUSIONS: We conclude that long-term microglial activation by LPS is not sufficient to drive dysfunction and neuronal death in organotypic hippocampal slice cultures, unless primed with IFNγ. Activation of iNOS and production of nitric oxide is suggested as a critical mediator of LPS/IFNγ toxicity.

Disclosures: I. Papageorgiou: None. **A. Lewen:** None. **T. Regen:** None. **U. Hanisch:** None. **O. Kann:** None.

Nanosymposium

198. Neuroinflammation in Alzheimer's Disease

Location: 143A

Time: Sunday, November 16, 2014, 1:00 PM - 3:45 PM

Presentation Number: 198.03

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

Support: DFG Excellence cluster SyNergy

Title: TREM2 mutations linked to neurodegeneration impair cell surface transport and phagocytosis

Authors: *C. HAASS;
DZNE (German Ctr. for Neurodegenerative Diseases), Munich, Germany

Abstract: Genetic variants in the triggering receptor expressed on myeloid cells 2 (TREM2) have been linked to Nasu-Hakola disease, Alzheimer’s disease (AD), Parkinson's disease, amyotrophic lateral sclerosis, frontotemporal dementia (FTD) and FTD-like syndrome without bone involvement. TREM2 is an innate immune receptor preferentially expressed in microglia and involved in inflammation and phagocytosis. Whether and how TREM2 missense mutations affect TREM2 function is elusive. Here we report that missense mutations associated with FTD and FTD-like syndrome reduce TREM2 maturation, abolish shedding by ADAM proteases and impair phagocytosis. An AD associated mutant TREM2 variant also reduces shedding although to a lower extent. As a consequence of reduced shedding TREM2 is virtually absent in the cerebrospinal fluid (CSF) and plasma of a patient with FTD-like syndrome. Lower levels of

TREM2 were also observed in CSF of AD and FTD patients further supporting that reduced TREM2 function may contribute to the risk for two prominent neurodegenerative disorders.

Disclosures: C. Haass: None

Nanosymposium

198. Neuroinflammation in Alzheimer's Disease

Location: 143A

Time: Sunday, November 16, 2014, 1:00 PM - 3:45 PM

Presentation Number: 198.04

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

Support: A.P. Giannini Foundation Postdoctoral Fellowship to F.F.R.

NIH R01 MH097268 to P.M.T.

NIH R01 AG040060 to P.M.T.

Title: Alzheimer’s disease risk variant in TREM2 affects ventricular expansion patterns in dementia and normal aging

Authors: *F. F. ROUSSOTTE[1], B. A. GUTMAN[2], D. P. HIBAR[2], S. K. MADSEN[2], P. M. THOMPSON[2];
[1]Neurol., UCLA, LOS ANGELES, CA; [2]USC, Los Angeles, CA

Abstract: Introduction We recently reported that elderly carriers of the rs939471 risk allele, a close proxy for a rare variant in TREM2 that triples the lifetime risk of Alzheimer’s disease (AD), annually lost up to 3.3% more brain tissue in the temporal lobes than noncarriers. That study did not control for dementia status. As this TREM2 variant is implicated in multiple neurodegenerative processes, here we hypothesized that the rs939471 risk allele would predict altered trajectories of lateral ventricular expansion, both in dementia and normal aging. Methods We tracked the volume of the lateral ventricles across baseline (N=736), 1-year (N=622), and 2-year (N=479) follow-up scans, in elderly participants from the Alzheimer’s Disease NeuroImaging Initiative. We used general linear models to determine if rs939471 genotype predicted ventricular expansion over a 2-year period, assuming an additive model of allele effects. Results At baseline, the rs939471 risk allele was not significantly associated with total ventricular volume (p=0.323), but it predicted larger volume of the left (p=0.043) but not the right ventricle (p=0.779), after controlling for age, sex, diagnosis, and ApoE status. At both follow-up points, different effects were identified. The same allele was significantly associated

with total ventricular expansion ($p=0.015$ and $p=0.002$ after 1 and 2 years, respectively) and with ventricular enlargement of the right ($p=0.001$ and $p<0.001$) but not the left ventricle ($p=0.129$ and $p=0.102$), after controlling for the same variables. Conclusion This is the first study to show that an AD risk variant in TREM2 is associated with altered trajectories of lateral ventricular enlargement in the elderly. As expected, the risk allele predicts larger ventricular volumes, but the mechanisms and laterality of its effects are unclear. Some have argued that the left hemisphere is affected earlier in AD and may be more susceptible to particular neurodegenerative processes. The largest genome-wide association study for CSF p-tau to date reports a strong association between the TREM2 risk variant and increased CSF p-tau levels, indicating neuronal death due to neurofibrillary tangles. Lateral ventricle expansion typically reflects hippocampal atrophy, and two recent studies suggest that the negative correlation between CSF p-tau levels and hippocampal volumes is left-lateralized. It is thus plausible that the rs939471 risk allele may have delayed effects on the right hemisphere via other, possibly slower neurodegenerative mechanisms, perhaps interfering with the anti-inflammatory and amyloid-β clearance functions of microglial cells expressing the TREM2 receptor.

Disclosures: **F.F. Roussotte:** None. **B.A. Gutman:** None. **D.P. Hibar:** None. **S.K. Madsen:** None. **P.M. Thompson:** None.

Nanosymposium

198. Neuroinflammation in Alzheimer's Disease

Location: 143A

Time: Sunday, November 16, 2014, 1:00 PM - 3:45 PM

Presentation Number: 198.05

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

Support: NINDS Grant NS084210

The Dana Foundation

Title: Adult human microglia proliferate in culture to high passage and maintain their response to the amyloid-β peptide

Authors: C. GEULA[1], A. REZVANIAN[1], M. PETERSON[1], T. GEFEN[1], S. WEINTRAUB[1], E. BIGIO[1], *M.-M. MESULAM[1], J. EL KHOURY[2], L. GUO[1];
[1]Norrthwestern Univ., Cognitive Neurol. and Alzheimer's Dis. Ctr., CHICAGO, IL; [2]Med. / Infectious Dis., Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA

Abstract: A great deal of what has been learned regarding microglial biology is based on *in vitro* studies the overwhelming majority of which have used cells isolated from the rodent brain. However, higher anatomical and functional complexity of the human brain and species differences in microglial response make imperative the use of human microglia to ascertain that the results obtained are applicable to man. Furthermore, investigation of microglial function in the adult brain, in which many inflammatory and anti-inflammatory microglial responses occur, requires use of adult human microglia. Microglia cultured from embryonic human brains show substantial proliferative capacity. However, while methods for isolation of microglia from adult postmortem human brains exist, they allow use of a limited quantity of microglia isolated and cultured from each case due to low levels of proliferation. We have developed a new technique which allows culturing microglia from postmortem adult human brains to high passage. Gray matter from cortical tissue in frontal poles of 5 cognitively normal aged individuals and 5 patients suffering from Alzheimer's disease (AD) was used. Dissociated cells were cultured in a medium containing microglia growth supplement and granulocyte macrophage colony stimulating factor. Microglia from both normal and AD cortex displayed excellent proliferation to high passage (20 passages, highest attempted). It took 7-10 days for the cells in each passage to reach 70-80% confluence. We did not observe differences in proliferation rate in cultures derived from tissue with different postmortem intervals up to 24 hours. Furthermore, we did not observe differences in rate of growth in cultures from normal brains when compared with brains from AD patients. Cryopreserved microglia displayed similar proliferation when compared with fresh cultures. Cultured cells of various passages displayed immunoreactivity for the specific microglia marker cluster of differentiation (CD)-68, but not for glial fibrillary acidic protein (GFAP), a specific marker of astrocytes. Nearly 100% of the cultured cells endocytosed acetyl low density lipoprotein (Ac-LDL), a ligand for scavenger receptors and a marker of microglia. Cultured microglia from normal and AD cortex produced substantial reactive oxygen species in response to fibrillar amyloid-β (Aβ) peptide, and significantly less in response to oligomeric Aβ. We did not detect differences in response to Aβ in cultures of different passages, nor between cultures from normal and AD brains. In conclusion, adult human microglia proliferate and survive to high passage in culture with maintained function.

Disclosures: **C. Geula:** None. **A. Rezvanian:** None. **M. Peterson:** None. **M. Mesulam:** None. **T. Gefen:** None. **S. Weintraub:** None. **E. Bigio:** None. **J. El Khoury:** None. **L. Guo:** None.

Nanosymposium

198. Neuroinflammation in Alzheimer's Disease

Location: 143A

Time: Sunday, November 16, 2014, 1:00 PM - 3:45 PM

Presentation Number: 198.06

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

Support: NIH AT006816

VA Merit (GMC, SAF)

Mary S Easton Consortium

Title: Targeting alzheimer-related innate immune gene expression with available therapeutics

Authors: *G. M. COLE[1], B. TETER[1], T. MORIHARA[2], Q. MA[1], X. ZUO[1], F. YANG[1], S. FRAUTSCHY[1];
[1]GRECC (VA) & Neurol/Med (UCLA), UCLA, VA Med. Ctr., Los Angeles, CA; [2]Psychiatry, Grad. Sch. of Med., Osaka Univ., Oskaa, Japan

Abstract: Background: GWAS and other studies implicate causal roles for innate immune microglial-expressed genes TREM2 and CD33 in Alzheimer Disease (AD) pathogenesis. To date, these studies support protective effects of TREM2 and deleterious effects of CD33. CD33 stimulates SHP tyrosine phosphatase activity opposing the TREM2/ TYROBP tyrosine kinase signaling that leads to CD68 positive phagocytes. CD33 opposes amyloid clearance while TREM2, like anti-abeta immunotherapies, promotes clearance, suggesting therapeutics that increase TREM2/TYROBP and decrease CD33 expression might reduce AD risk.**Methods:** APPsw Tg2576 transgenic mice were treated with immunomodulatory dietary curcumin from 10 to 16 months of age. Amyloid plaque burden and microglial phosphotyrosine (PT) were quantified by ICC while insoluble Abeta and IL-1beta were measured by ELISA. Cortical mRNA for TREM2, TYROBP, CD33, CD68, CD11b, iNOS and Arg-1 were measured by real time qPCR. Curcumin was also used in vitro with primary rodent microglia and cell lines, including human THP-1. **Results:** Dietary low dose curcumin significantly reduced amyloid and increased pro-phagocytic TREM2, TYROBP and CD68 but reduced CD33, microglial marker CD11b and M1-related iNOS and IL-1beta protein. TREM2 co-localized with elevated peri-plaque PT-labeled microglia. TREM2 and TYROBP mRNA levels correlated positively with peri-plaque PT in curcumin treated mice. High dose curcumin significantly reduced M2 marker Arg-1 and failed to lower amyloid or increase the M2 marker TREM2. In vitro, low dose curcumin directly reduced CD33 and increased TREM2 protein levels in rodent and human cell lines. Low dose curcumin increased amyloid phagocytosis and clearance from AD brain sections and increased microglial phagocytosis of beads. **Conclusions:** TREM2 and TYROBP expression are central hubs controlling AD gene expression changes. Our results show that dietary curcumin acts as an immunomodulator by reducing expression of new AD innate immune target gene product CD33 while increasing dementia protective TREM2 expression consistent with increased amyloid clearance and anti-inflammatory activity in vitro and in vivo. These effects are seen with human myeloid lineage cells in vitro and with drug levels that are achievable in

patients with new formulations of curcumin currently in clinical trials at our site. We conclude that curcumin is a strong candidate for controlling innate immune expression related to AD risk.

Disclosures: **G.M. Cole:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); UCLA/VA patent on curcumin formulation Longvida licensed to Verdure Biosciences. **B. Teter:** None. **T. Morihara:** None. **Q. Ma:** None. **X. Zuo:** None. **F. Yang:** None. **S. Frautschy:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); UCLA/VA patent on curcumin formulation Longvida licensed to Verdure Biosciences.

Nanosymposium

198. Neuroinflammation in Alzheimer's Disease

Location: 143A

Time: Sunday, November 16, 2014, 1:00 PM - 3:45 PM

Presentation Number: 198.07

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

Support: Cure Alzheimer's Fund

NIH Grant F32-NS083187-01A1

NMSS JF 2144A2/1

Title: Altered microglial response to Abeta plaques in APPPS1-21 mice heterozygous for TREM2

Authors: ***J. D. ULRICH**[1], M. FINN[1], Y. WANG[2], A. SHEN[1], T. E. MAHAN[1], H. JIANG[1], F. R. STEWART[1], L. PICCIO[1], M. COLONNA[2], D. M. HOLTZMAN[1];
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Abstract: Recent genome-wide association studies linked variants in TREM2 to a strong increase in the odds of developing Alzheimer’s disease, however the mechanism by which TREM2 influences the susceptibility to Alzheimer’s disease is currently unknown. Within the brain, TREM2 is expressed by microglia and is thought to regulate microglial phagocytic and inflammatory responses to pathological insults. Since a single allele of variant TREM2, that is hypothesized to be detrimental to TREM2 function, conferred an increased risk of developing Alzheimer’s disease, we tested whether loss of one functional trem2 allele would affect Aβ plaque deposition or the microglial response to Aβ pathology in APPPS1-21 mice. We observed

no significant difference in Aβ deposition in 3-month old or 7-month old APPPS1-21 mice expressing one or two copies of trem2. However, 3-month old mice with one copy of trem2 exhibited a marked decrease in the size and number of microglia associated with Aβ plaques. While there was no statistically significant differences in cytokine levels or markers of microglial activation in 3- or 7-month old animals, there were trends towards decreased expression of NOS2, C1qa, and IL1a in 3-month old TREM2+/- vs. TREM2+/+ mice. Therefore, we found that loss of a single copy of trem2 had no effect on Aβ deposition, but altered the morphological phenotype of plaque-associated microglia. These data suggest that TREM2 regulates the microglial response to Aβ deposition but does not affect Aβ plaque burden.

Disclosures: **J.D. Ulrich:** None. **M. Finn:** None. **Y. Wang:** None. **T.E. Mahan:** None. **H. Jiang:** None. **F.R. Stewart:** None. **L. Piccio:** None. **M. Colonna:** None. **D.M. Holtzman:** None. **A. Shen:** None.

Nanosymposium

198. Neuroinflammation in Alzheimer's Disease

Location: 143A

Time: Sunday, November 16, 2014, 1:00 PM - 3:45 PM

Presentation Number: 198.08

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

Support: NIH RO1AG030209

NIH R21AG033914

Alzheimer's Association

American Federation for Aging Research

Swedish Research Council

NIH NRSA F31AG039195

NIH NRSA 1F31NS074712

Title: Microglial deletion of the PGE2 receptor EP2 restores immune and trophic responses and rescues cognitive function in Alzheimer’s disease models

Authors: ***K. I. ANDREASSON**[1], J. JOHANSSON[1], N. WOODLING[1], X. LIANG[1], Q. WANG[1], H. BROWN[1], M. PANCHAL[1], T. LOUI[1], A. TRUEBA-SAIZ[2], S. PRADHAN[1];

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Abstract: Microglia, the innate immune cells of the central nervous system, perform critical inflammatory and non-inflammatory functions to maintain local homeostasis and normal neural function. However in Alzheimer's disease (AD), these beneficial functions become progressively impaired, contributing to dysregulated and toxic inflammatory responses, synaptic and neuronal loss, and ultimately cognitive impairment. The inflammatory cyclooxygenase-PGE_2 pathway has been implicated in pre-clinical AD development, both in human epidemiology and in transgenic murine models of AD. In our studies using in vitro and in vivo conditional knockout approaches, we have determined that in mouse models of AD, cell-specific deletion of the microglial PGE_2 EP2 receptor restores microglial chemotaxis and Aß-clearance activity, promotes resolution of toxic inflammatory responses, and increases expression of cytoprotective insulin-like growth factor 1 and Akt signaling. We also find that cell-specific ablation of microglial EP2 prevents onset of hippocampal-dependent memory deficits in AD model mice. In human cerebral cortex, microglial EP2 receptor levels increase as subjects progress from normal aging to mild cognitive impairment to AD. In its overall regulation of distinct microglial functions, our findings indicate that EP2 signaling is a general suppressor of multiple beneficial processes that falter in microglia in the development of AD pathology. Inhibition of inflammatory EP2 signaling may represent a novel approach to restore healthy microglial function that can prevent and delay the development of AD.

Disclosures: K.I. Andreasson: None. **J. Johansson:** None. **N. Woodling:** None. **X. Liang:** None. **Q. Wang:** None. **H. Brown:** None. **M. Panchal:** None. **T. Loui:** None. **S. Pradhan:** None. **A. Trueba-Saiz:** None.

Nanosymposium

198. Neuroinflammation in Alzheimer's Disease

Location: 143A

Time: Sunday, November 16, 2014, 1:00 PM - 3:45 PM

Presentation Number: 198.09

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

Support: NIH grant NS079637

NIH grant P20GM103486

NCRR 5P20RR020171

Title: Determining the role of an M2a phenotype on microglial activity and amyloid deposition using BV2 microglial cells and APP/PS1 transgenic mice

Authors: *C. H. LATTA[1], T. L. SUDDUTH[1], E. M. WEEKMAN[2,1], H. M. BROTHERS[1], F. GONZALEZ OREGON[1], K. J. BRAUN[1], D. M. WILCOCK[2,1];
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Abstract: Microglia are considered to be the resident macrophages of the brain. In their resting state, microglia extend ramified process that probe the brain parenchyma for pathogenic activity and damage. In response to detrimental stimuli, a course of inflammation governs a polarized spectrum of microglial phenotypes based on peripheral macrophage profiles; M1, M2a, M2b and M2c. Classically activated microglia, M1, express pro-inflammatory cytokines as well as oxygen and nitrogen radicals. This phase is frequently termed "a double edged sword"; a toxic environment eradicates any pathogenic activity, yet is destructive to nervous tissue. The transition to an alternative state, M2, establishes a habitable environment permitting repair and regeneration. An M2a phenotype increases the production of anti-inflammatory cytokines and extracellular matrix remodeling proteins consequently dampening the pro-inflammatory response and aiding wound healing. This study aimed to determine the effect of an M2a phenotype on Alzheimer's disease (AD) pathological progression with in vitro and in vivo models. Our approach is to define the inflammatory state of microglia in response to external stimuli such as cytokine application. IL-4 is a strong M2a polarizing cytokine in macrophages but is not secreted by microglia. To initially characterize an M2a phenotypic change in microglia, we used BV2 microglial cells to study the temporal progression of microglial responses to IL-4. The BV2 cells were incubated in serum-free DMEM/F12 media containing murine IL-4. To assess the effect of the released factors on the hallmark pathologies of AD, media was extracted after 8 hours of incubation ,the optimal M2a state, and transferred to CHO APP cells (secreting β-amyloid) and Hek WT Tau and P301L cells (expressing human wild type tau and a tau variant respectively). Additionally, we intracranially injected an adeno-associated viral (AAV) vector to express IL-4 in the frontal cortex and hippocampus of APP/PS1 transgenic mice (which overexpress β-amyloid) at 3 months of age and we evaluated the mice 6 weeks post-injection. Quantitative real-time PCR was used to assess biomarker expression of microglial phenotypes in both the animal tissue and the BV2 cells. Protein analysis was performed on the brain tissue and cell cultures to quantify β-amyloid and tau depositions. Histological staining permitted quantification of microglial activity. Both models showed enhanced M2a phenotypic expression and IL-4 treatment revealed a trend of decreased β-amyloid in the animal models. In summary, this study offers insight into the therapeutic potential of modulating microglial immune response in AD.

Disclosures: C.H. Latta: None. **T.L. Sudduth:** None. **E.M. Weekman:** None. **H.M. Brothers:** None. **F. Gonzalez Oregon:** None. **K.J. Braun:** None. **D.M. Wilcock:** None.

Nanosymposium

198. Neuroinflammation in Alzheimer's Disease

Location: 143A

Time: Sunday, November 16, 2014, 1:00 PM - 3:45 PM

Presentation Number: 198.10

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

Support: NIA Grant AG023012

NINDS Grant NS047804

NINDS Grant NS087298

DOD Grant W81XWH12-1-0629

Bright Focus Foundation A2013252F

NRSA T32 NS067431

A generous donation from Chet and Jane Scholtz

Title: Trem2 expression is upregulated in myeloid cells surrounding plaques in Alzheimer's disease and Trem2 deficiency modulates pathology

Authors: *T. R. JAY[1,2,5], C. M. MILLER[2], L. C. GRAHAM[5], S. BEMILLER[2], C. KARLO[1], G. XU[2], S. STAUGAITIS[2], L. BEKRIS[3], J. LEVERENZ[4], G. HOWELL[5], R. RANSOHOFF[2], G. LANDRETH[1], B. T. LAMB[2];
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Abstract: Alzheimer's disease (AD) is characterized by extracellular accumulation of beta amyloid (Aβ), intraneuronal accumulation of microtubule associated protein tau (MAPT), and by aberrant neuroinflammation. The integral role of inflammation in AD pathogenesis was highlighted by recent studies which showed that mutations in Trem2, an important modulator of myeloid cell activation, confer high risk for developing AD. Using RNA and protein analyses, we found that Trem2 expression is upregulated in three mouse models of Alzheimer's disease and in human AD tissue. Immunohistochemistry and Trem2lacZ/lacZ knock-in mice revealed that the upregulation of Trem2 is localized within Aβ plaque-associated myeloid cells. Our data also suggest that loss of Trem2 in these AD mouse models modulates Aβ and MAPT-related

pathologies. These data explore a possible pathogenic mechanism underlying Trem2 mutations, which will be important to more fully understand the role of inflammation in AD.

Disclosures: **T.R. Jay:** None. **C.M. Miller:** None. **S. Bemiller:** None. **G. Xu:** None. **C. Karlo:** None. **L. Bekris:** None. **S. Staugaitis:** None. **J. Leverenz:** None. **G. Landreth:** None. **G. Howell:** None. **R. Ransohoff:** None. **B.T. Lamb:** None. **L.C. Graham:** None.

Nanosymposium

198. Neuroinflammation in Alzheimer's Disease

Location: 143A

Time: Sunday, November 16, 2014, 1:00 PM - 3:45 PM

Presentation Number: 198.11

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

Support: Jane and Lee Seidman Fund

NIA AG023012 to B.T.L.

NINDS NS047804 to B.T.L.

NINDS NS087298 to B.T.L. and R.M.R.

DOD W81XWH12-1-0629 to B.T.L.

BrightFocus Foundation A2013252F to C.M.M.

NRSA T32 NS067431 to T.R.J.

Title: Trem2 is predominantly expressed by infiltrating monocytes in multiple mouse models of Alzheimer’s disease

Authors: *C. M. MILLER[1], T. R. JAY[1,2], L. C. GRAHAM[3], A. COTLEUR[1], G. LANDRETH[2], G. HOWELL[3], R. M. RANSOHOFF[1], B. T. LAMB[1,2];
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Abstract: Mutations in triggering receptor expressed on myeloid cells 2 (Trem2) were recently shown to confer high risk for developing Alzheimer’s disease (AD) as well as other neurodegenerative diseases. It was anticipated that Trem2 would be expressed predominantly on microglia, the brain-resident myeloid cell population. To test this hypothesis and determine

which myeloid cells in the brain express TREM2, flow cytometry was performed in three different mouse models of AD followed by RNA sequencing. Interestingly, there was an age-dependent increase in the percentage of TREM2-expressing CD11b+/CD45^{hi} peripheral monocytes versus CD11b+/CD45^{lo} microglia. Additional flow and immunohistochemical labeling with F4/80 and Ly6C further established macrophage lineage and monocytic cell identity, respectively. These data suggest that Trem2 is predominantly expressed on macrophages derived from peripheral monocytes in mouse models of AD. Taken together, these results indicate that examining peripheral monocytes will be integral to understanding how Trem2 mutations contribute to the pathogenesis of AD and further delineate the divergent roles of microglia and monocytes.

Disclosures: C.M. Miller: None. **T.R. Jay:** None. **L.C. Graham:** None. **A. Cotleur:** None. **G. Landreth:** None. **G. Howell:** None. **R.M. Ransohoff:** None. **B.T. Lamb:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.01

Topic: C.03. Parkinson’s Disease

Support: Michael J. Fox Foundation

Title: Cerium oxide nanoparticles as a disease-modifying therapy for Parkinson's disease

Authors: A. S. FREY[1], B. LOCKLER[1], M. J. BILLINGS[1], K. S. HOCKEY[1], C. A. SHOLAR[1], *B. A. RZIGALINSKI[2];
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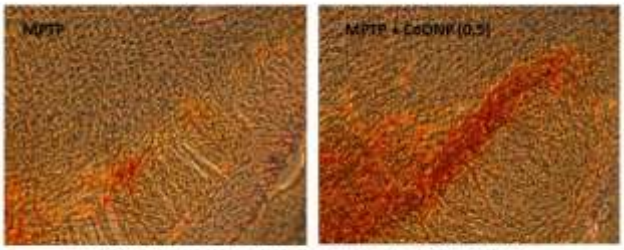
Abstract: Cerium oxide nanoparticles (CeONP) are highly efficient mitochondrial protectants and regenerative free radical scavengers. Our prior work in the MPTP-mouse model of Parkinson's disease, found that administration of CeONP prior to MPTP challenge could completely protect mice from dopaminergic loss. In the present work, we tested the hypothesis that CeONP may be a disease-modifying therapy for Parkinson’s disease, when delivered after development of the disease. C57Bl/6 mice were treated with 20 mg/kg MPTP given in 4 injections spaced 2 hrs apart. CeONP (0.05-5.0 micrograms/g) was delivered intravenously in a single dose, 12 hrs after the last MPTP injection. Seven days later, mice were euthanized and dopamine content in the striatum was measured. Dopaminergic neurons in the substantia nigra

were stained and sterotaxically counted. Lipid peroxidation levels in the brain were also measured. We found that: • CeONP increased the levels of TH+ neurons in the substantia nigra, when delivered alone (No MPTP) • CeONP preserved striatal dopamine by approximately 50%, when delivered after development of the disease. • CeONP preserved dopaminergic neurons in the substantia nigra to 84-87% of controls when delivered after development of the disease • CeONP decreased basal levels of lipid peroxidation in the cortex These results suggest that CeONP may halt or slow disease progression. Further, the ability of CeONP to promote growth of neurons in the substantia nigra suggests the potential to reverse



CeONP preserves dopaminergic neurons in the striatum of MPTP-treated mice when delivered 12 hrs after MPTP challenge.

PD.

Disclosures: **A.S. Frey:** None. **B. Lockler:** None. **M.J. Billings:** None. **K.S. Hockey:** None. **C.A. Sholar:** None. **B.A. Rzigalinski:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.02

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Michael J. Fox Foundation

NIH Grant P01 ES016738

NIH Grant P01 HD29587

NIH Grant P30 NS076411

Title: Transnitrosylation from DJ-1 to PTEN attenuates neuronal cell death in Parkinson's disease models

Authors: *T. NAKAMURA[1], M.-S. CHOI[1], S.-J. CHO[2], E. A. HOLLAND[1], J. QU[1], G. A. PETSKO[3], R. C. LIDDINGTON[2], S. A. LIPTON[1];
[1]Neurosci. and Aging Res. Ctr., [2]Program on Infectious Dis., Sanford-Burnham Med. Res. Inst., LA JOLLA, CA; [3]Weill Cornell Med. Col., New York, NY

Abstract: Emerging evidence suggests that oxidative/nitrosative stress, as occurs during aging, contributes to the pathogenesis of Parkinson's disease (PD). In contrast, detoxification of reactive oxygen and nitrogen species (ROS/RNS), can protect neurons. DJ-1 has been identified as one of several recessively-inherited genes whose mutation can cause familial PD, and inactivation of DJ-1 renders neurons more susceptible to oxidative stress and cell death. DJ-1 is also known to regulate phosphatase and tensin homolog (PTEN) activity, which plays a critical role in neuronal cell death in response to various insults. However, mechanistic details delineating how DJ-1 regulates PTEN activity remain unknown. Here, we report that PTEN phosphatase activity is inhibited via a transnitrosylation reaction, i.e., transfer of an NO group from the cysteine residue of one protein to another. Specifically, we show that DJ-1 is S-nitrosylated (forming SNO-DJ-1); subsequently, the NO group is transferred from DJ-1 to PTEN by transnitrosylation. Moreover, we detect S-nitrosylated (SNO)-PTEN in human brains of sporadic PD. Using X-ray crystallography and site-directed mutagenesis, we find that Cys106 is the site of S-nitrosylation on DJ-1 and mutation of this site inhibits transnitrosylation to PTEN. Importantly, S-nitrosylation of PTEN decreases its phosphatase activity, thus promoting cell survival. These findings provide mechanistic insight into the neuroprotective role of SNO-DJ-1 by elucidating how DJ-1 detoxifies NO via transnitrosylation to PTEN. Dysfunctional DJ-1, which lacks this transnitrosylation activity due to mutation or prior oxidation (e.g. sulfonation) of the critical cysteine thiol, could thus contribute to neurodegenerative disorders like PD.

Disclosures: T. Nakamura: None. **M. Choi:** None. **S. Cho:** None. **E.A. Holland:** None. **J. Qu:** None. **G.A. Petsko:** None. **R.C. Liddington:** None. **S.A. Lipton:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.03

Topic: C.03. Parkinson's Disease

Support: MJ Fox Foundation

Title: Fractalkine over expression suppresses α-synuclein mediated neurodegeneration

Authors: *K. R. NASH[1], D. MORGAN[2], P. C. BICKFORD[2];
[1]Mol. Pharmacol. and Physiol., Univ. of South Florida, Tampa, FL; [2]USF, Tampa, FL

Abstract: In Parkinson's disease (PD) α-synuclein activates microglia and this activation has been suggested as one of the mechanisms of neurodegeneration. There are several signals produced by neurons that have an anti-inflammatory action on microglia, including CX3CL1 (fractalkine). We have previously shown that a soluble form of CX3CL1 is required to reduce neuron loss in MPTP treated mice and that fractalkine agonism can reduce neuron loss in a 6-hydroxydopamine lesion model. Here we show that fractalkine can reduce α-synuclein mediated neurodegeneration in rats. Rats that received fractalkine showed abrogated loss of tyrosine hydroxylase and Neu-N staining. This was replicated in animals where we expressed fractalkine from astrocytes with the GFAP promoter. Interestingly, we did not observe a rescue of neuron loss with the membrane associated form of fractalkine. Further, we did not observe a reduction in MHCII expression suggesting that soluble fractalkine is likely altering the microglial state to a more neuroprotective one rather than reducing antigen presentation. We report that fractalkine receptor agonism with the soluble FKN can rescue neuron loss in the recombinant adeno-associated virus (rAAV) mediated α-synuclein model of PD and warrants further investigation as a therapeutic target.

Disclosures: K.R. Nash: None. **D. Morgan:** None. **P.C. Bickford:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.04

Topic: C.03. Parkinson's Disease

Support: NIH RO1 Grant NS70898

Title: MicroRNA-7 targets RelA to improve cellular bioenergetics and protect against MPP+-induced cell death

Authors: A. DATTA CHAUDHURI[1], S. KABARIA[2], D.-C. CHOI[1], M. MOURADIAN[2], *E. JUNN[3];

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Abstract: Mitochondrial dysfunction and aberrant cellular bioenergetics are hallmarks of Parkinson's disease (PD). These pathological features of the disease can be mimicked in vitro by treating dopaminergic cells with the mitochondrial complex I inhibitor 1-methyl-4-phenylpyridinium (MPP+). In this paradigm, as oxidative phosphorylation is blocked, cells become dependent on glycolysis to meet their energy demands. Increasing the rate of glycolysis can, therefore, rescue cells from MPP+-induced death. MicroRNA-7 (miR-7) is a small, non-coding RNA that is protective in PD models by reducing α-synuclein expression. In the present study, we show that miR-7 also protects against MPP+-induced cytotoxicity. Overexpression of miR-7 in SH-SY5Y cells and differentiated ReNCell VM cells (mesencephalic neural progenitor cell line) prevented cell death and loss of neurites, respectively, induced by MPP+. This protective effect of miR-7 was mediated through down-regulating its target mRNA RelA. Knocking down RelA with siRNA had a similar protective effect. Considering that RelA knockdown increases the rate of glycolysis, we sought to examine whether the mechanism of cytoprotection provided by miR-7 also involves an increase in glycolytic rate. Treatment of SH-SY5Y cells with MPP+ resulted in an expected decrease in ATP production attributed to loss of complex I activity. On other hand, overexpression of miR-7 or knockdown of RelA augmented the rate of glycolysis as evidenced by an in increase in ATP production, glucose consumption and lactate production. Furthermore, both miR-7 and siRelA failed to protect against MPP+-induced cell death when cells were cultured in a low glucose (1 g/L) media instead of regular media containing 4.5 g/L glucose. The latter finding indicates that availability of the glycolytic substrate is required for the observed protective effect. We can, therefore, conclude that miR-7, through down-regulating RelA, promotes glycolysis in order to sustain energy production in the absence of complex I activity and protects against the cytotoxic effect of MPP+.

Disclosures: **A. Datta Chaudhuri:** None. **E. Junn:** None. **D. Choi:** None. **S. Kabaria:** None. **M. Mouradian:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.05

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Deutsche Forschungsgemeinschaft

Title: Influence of the Ca2+-independent phospholipase A2 (iPLA2) and docosahexaenoic acid on the electron-transport-chain dependent ROS generation in brain mitochondria

Authors: *G. REISER, C. NORDMANN, P. SCHÖNFELD;
Otto-von-Guericke Univ., D39120 Magdeburg, Germany

Abstract: Drug-based prevention of reactive oxygen species (ROS) generation is important, because oxidative stress is a factor for the pathogenesis of many diseases, like neurodegeneration in Alzheimers disease and many others. The Ca2+-independent phospholipase A2 (iPLA2) liberates free fatty acids (FFA) by hydrolyzing the sn-2 ester bond of membrane glycerophospholipids. iPLA2 has been found in various mammalian mitochondria. According to a current concept, the activity of the inner mitochondrial membrane-associated iPLA2 removes oxidatively damaged fatty acids for lipid remodeling and repair. Mitochondria are main ROS producers in cells. Thus, the question arises, whether iPLA2 has antioxidative defense, and attenuates the formation of electron transport chain (ETC)-associated superoxide (O2•-), and thereby reduces oxidative stress. Mild-uncoupling is a suggested mechanism. We examined the influence of iPLA2 on ETC-associated ROS generation in rat brain mitochondria (RBM). First, we used docosahexanoic acid (DHA). DHA is a major reaction product of iPLA2, and was used to adjust mild-uncoupling in succinate-oxidizing RBM and, to impair reversed electron transport (RET) in ETC. We find that low DHA concentrations diminish RET-dependent ROS generation by mild-uncoupling. This was largely due to adenine nucleotide translocase. However, when mitochondria oxidize glutamate plus malate and, thereby support the forward electron transport (FET), DHA enhanced mitochondrial ROS generation. In addition, to reduce the endogenous mitochondrial pool of FFA, mitochondria were treated with the specific iPLA2-inhibitor bromoenol lactone (BEL). BEL-treated (succinate-oxidizing) mitochondria show enhanced ROS generation, but are slightly depolarized in comparison to untreated control. This contradicts the view that iPLA2 inhibition abolishes mild uncoupling and, consequently, enhances the mitochondrial membrane potential. Our novel hypothesis is that we explain mechanistically the increase of the ROS release by BEL-treated RBM with a diminished content of reduced glutathione. Thus, we disprove the concept that iPLA2 attenuates oxidative stress in brain mitochondria. Further work will elucidate mechanisms of pathogenesis of neurodegenerative disorders based on the dysregulated iPLA2. We anlyse this question in a genetic mouse model of INAD: Strokin, M., Seburn, K.L., Cox, G.A., Martens, K.A., Reiser, G.,. Severe disturbance in the Ca2+ signaling in astrocytes from mouse models of human infantile neuroaxonal dystrophy (INAD)with mutated Pla2g6. Hum. Mol. Genet. 21, 2012, 2807-2814

Disclosures: **G. Reiser:** None. **P. Schönfeld:** None. **C. Nordmann:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.06

Topic: C.03. Parkinson’s Disease

Support: Midwestern University Intramural Funds

Title: Exploring the effects of nicotine on NADH dehydrogenase activity and mitochondrial morphology in a *Drosophila* model of Parkinson's disease

Authors: *L. M. BUHLMAN[1], M. ODUMOSU[1], G. B. CALL[2];
[1]Biomed. Sci., [2]Arizona Col. of Med., Midwestern Univ., Glendale, AZ

Abstract: NADH dehydrogenase (mitochondrial respiratory chain complex 1) deficiency is implicated in both sporadic and genetic forms of Parkinson's disease (PD). Mitochondrial toxins that inhibit electron transport from complex 1 are widely used to created sporadic models of PD, while patients with autosomal recessive-juvenile parkinsonism caused by mutations in PARK2 exhibit decreased complex 1 activity as measured in cultured fibroblasts. Nicotine pretreatment has been shown to be beneficial in sporadic PD models, and it can increase median lifespan and improve motor and olfactory deficits in Parkin loss-of-function Drosophila. The mechanism by which nicotine offers protection against the mutant phenotype in Drosophila is unclear. Because nicotine has been shown to bind to complex 1 and affect its activity, we hypothesize that nicotine ameliorates the Parkin loss-of-function phenotype by restoring normal function of complex 1. To this end, we performed in-gel activity assays on mitochondrial fractions from heads of adult Drosophila raised on nicotine and found that nicotine has different effects on complex 1 in mutants compared to control flies. Because Parkin-loss-of-function affects mitochondrial fission events required for disposal of poorly-functioning mitochondria (mitophagy), we explored the possibility that our mutant flies would exhibit aberrant mitochondrial morphology and turnover, which could be particularly detrimental for cells containing mitochondria with complex 1 deficits. Thus, we measured mitochondrial morphology and total mass per cell in TH-expressing neurons of adult Parkin loss-of-function Drosophila brains. Our results shed light on whether aberrant mitochondrial morphology and complex I function play a role in the manifestation of parkinsonism and whether nicotine exerts protective effects by restoring mitochondrial morphology and function.

Disclosures: L.M. Buhlman: None. **M. Odumosu:** None. **G.B. Call:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.07

Topic: C.03. Parkinson's Disease

Support: NIH Grant R01AG038961

NIH Grant R01EB009041

Title: Neuroprotective effects induced by focused ultrasound-facilitated AAV-GDNF delivery in a Parkinson's-disease mouse model

Authors: *S. WANG[1], O. OLUMOLADE[2], V. JACKSON-LEWIS[3], J. BLESA[3], T. SUN[2], G. SAMIOTAKI[2], S. PRZEDBORSKI[3], E. KONOFAGOU[2];
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Abstract: The pathology of Parkinson's Disease (PD) is characterized by the relatively selective death of nigro-striatal dopaminergic neurons. Utilizing recombinant adeno-associated virus (rAAV), therapeutic genes can be delivered to the brain for long-lasting treatments. However, the existence of the blood-brain barrier (BBB) prevents efficient delivery of the systemically administered viral vectors. Transcranial focused Ultrasound (FUS) in combination with microbubbles (MB) has been shown capable of inducing reversible blood-brain barrier (BBB) opening. In this study, we investigate the neuroprotective effects of non-invasively delivered rAAV-GDNF vectors after FUS induced BBB opening in a PD mouse model. Animals were divided into four groups (n = 4-6 per group): control, FUS only, rAAV injection only, and rAAV+FUS. For the FUS only and AAV+FUS groups, both striatum (Str) and substantia nigra (SN) were sonicated unilaterally at 1.5 MHz. For the rAAV+FUS group, immediately before sonication, a 100 μl mixture of rAAV1-CAG-hGDNF-GFP vectors (8.5×1011 GC/animal) and in-house polydispersed microbubbles (~2.5×107 #/animal) were administered IV. After 4-week survival, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was administered at 30 μg/kg IP over five consecutive days. Twenty-seven days after the last MPTP injection, the mice were sacrificed and their brains were prepared for tyrosine hydroxylase (TH) staining for subsequent TH-positive neuronal counting in SN. The optical density (OD) and the integrated OD (IOD) were quantified in MATLAB (Mathworks). The ratio of ipsilateral (FUS treated side) to contralateral side with TH-positive neurons in the AAV+FUS group was significantly higher ($p=0.03$) compared to all other groups. The OD and IOD of TH levels in the caudate-putamen was only statistical significant in the AAV+FUS group ($p=0.0059$ and $p=0.0063$, respectively)

compared to all other groups. The IOD of the TH level in the caudate-putamen further confirmed the neuroprotective effects of the non-invasively delivered rAAV-GDNF vectors FUS in combination with MB provide a non-invasive and targeted approach for gene delivery to specific brain targets. This study, for the first time, demonstrated neuroprotective effects of non-invasively delivered rAAV1-GDNF using transcranial FUS in a PD animal model.

Disclosures: **S. Wang:** None. **O. Olumolade:** None. **V. Jackson-Lewis:** None. **J. Blesa:** None. **T. Sun:** None. **G. Samiotaki:** None. **S. Przedborski:** None. **E. Konofagou:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.08

Topic: C.03. Parkinson’s Disease

Support: American Parkinson Disease Association

Parkinson's Association of Alabama

Title: 14-3-3s regulate alpha-synuclein release and toxicity

Authors: *T. A. YACOUBIAN, B. WANG;
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Abstract: Alpha-synuclein (αsyn) plays a critical role in Parkinson’s disease. Recent research suggests a prion-like mode for syn toxicity: αsyn is released as aggregated species that cause further aggregation and toxicity in neighboring cells. We have been investigating the role of the 14-3-3 proteins in regulating alpha-synuclein release and paracrine toxicity. 14-3-3s are chaperone-like proteins that reduce protein aggregation, regulate protein secretion, and promote cell survival. To examine the effect of 14-3-3s on αsyn release, we created a doxycycline (doxy)-inducible αsyn neuroblastoma line (isyn) that upon doxy treatment releases αsyn into conditioned media (CM) that is toxic to separately-cultured primary neurons. We observed that 14-3-3θ overexpression (OE) in isyn cells increased the total amount of αsyn released into CM by 3-fold compared to control isyn cells upon doxy induction for 96 hours. This increase in αsyn release with 14-3-3θ OE was noted as early as 48 hours and maintained at all time points examined up to 7 days. Fractionation of CM into exosomal and non-exosomal fractions using high ultracentrifugal spins revealed that αsyn levels were increased in exosomes but not in the non-

exosomal fraction with 14-3-3θ OE in isyn cells. Despite the increase in αsyn release, we observed a complete elimination of the toxicity of αsyn-enriched CM on separately cultured differentiated SH-SY5Y cells or primary hippocampal neurons. Conversely, 14-3-3 inhibition with the pan-14-3-3 inhibitor difopein caused a 40% decrease in αsyn release into the CM compared to control. This reduction in release was observed primarily in the exosomal fraction but was also seen in the non-exosomal fraction. Difopein in the isyn cells increased the toxicity of αsyn-enriched CM on target cells. To test whether 14-3-3θ OE affects the amount of monomeric vs. oligomeric αsyn released by cells, we used a bioluminescent protein-fragment complementation assay in which luciferase signal is generated when αsyn fused to a non-bioluminscent amino terminal luciferase fragment (S1) interacts with αsyn fused to a carboxy-terminal luciferase fragment (S2). The amount of luciferase signal was significantly reduced with 14-3-3θ OE in H4 cells transfected with S1-syn and S2-syn. Conversely, difopein increased luciferase signal compared to control. Based on these findings, we conclude that 14-3-3θ can regulate the release and toxicity of αsyn and may serve as a target for therapeutic intervention in Parkinson's disease.

Disclosures: **T.A. Yacoubian:** None. **B. Wang:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.09

Topic: C.03. Parkinson's Disease

Support: Dartmouth SYNERGY K12 Program-Early Investigators

Title: Prodromal anti-inflammatory intervention blocks leukocyte infiltration and the progression of Parkinsonism in rotenone treated mice

Authors: *M. C. HAVRDA;
Geisel Sch. of Med. at Dartmouth, Lebanon, NH

Abstract: In the Farming and Movement Evaluation Study, led by the National Institute of Environmental Health Sciences (NIEHS), exposure to the metabolic toxin rotenone, a broad-spectrum pesticide used in agriculture, has been identified as a risk factor for the development of Parkinson's disease. We exposed mice to low doses of rotenone orally, using intragastric gavage, 5 days per week, from 6-12 months of age. Using this model system, we observed classical

behavioral and histopathologic symptoms of Parkinsonism that developed progressively over a six month time period. To determine if rotenone caused inflammatory changes in the CNS, we evaluated freshly prepared brain cells from mice that had been exposed to rotenone or vehicle for 6 months. We observed the expected CD45lo (resident microglia) and rare CD45hi (peripheral leukocyte) populations in our preparations and found that rotenone specifically increased the percentages of CD45hi cells as compared to vehicle treated mice. Although we observed evidence of microglial activation in histologic samples, co-labeling for the monocyte and microglial marker CD11b along with CD45 indicated that there was no change in the overall percentages of CD45lo/CD11b+ cells in rotenone treated mice, however, significant increases in CD45hi/CD11b- cells were observed as the result of rotenone exposure. We concluded that rotenone exposure led to an infiltration of peripheral, non-monocytic leukocytes into the CNS. We obtained striatal tissue extracts from an independent cohort of mice that had only been exposed to rotenone for 3 months, prior to the development of behavioral symptomology. We conducted an unbiased cytokine screen and observed a significant induction of pro-inflammatory cytokines in the brains of rotenone treated animals. Administration of the anti-inflammatory phosphodiesterase 4 - inhibitor rolipram at the 3-month time point inhibited the development of motor symptoms. Post-mortem analysis revealed a complete inhibition of leukocyte infiltration into the CNS of rotenone treated mice receiving rolipram. These findings indicate that detectable, reversible neuroinflammation can occur as a result of occupational exposure to pesticides. Findings will inform the development of preventative anti-inflammatory treatments for neurologic disorders in at risk populations such as the elderly, agricultural workers and military personnel.

Disclosures: M.C. Havrda: None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.10

Topic: C.03. Parkinson’s Disease

Title: Caspase inhibition mitigates alpha-synuclein cytotoxicity and mitochondrial demise in human dopaminergic neurons

Authors: *G. K. GANJAM[1], A. M. DOLGA[1], K. BOLTE[2], S. NEITEMEIER[1], M. HÖLLERHAGE[3], W. E. OERTEL[4], G. U. HOEGLINGER[3], C. CULMSEE[1];

[1]Philipps Univ. of Marburg, Marburg, Germany; [2]Cell Biol., Dept. of Biol., Marburg, Germany; [3]German center for Neurodegenerative Dis., Munich, Germany; [4]Dept. of Neurol., Marburg, Germany

Abstract: Parkinson's disease is a common neurodegenerative movement disorder characterized by dopaminergic neuronal loss in the substantia nigra that has been linked to α-synuclein toxicity. The molecular mechanisms underlying α-synuclein accumulation, agglomeration and toxicity in human dopaminergic neuronal loss are poorly defined. Hence, the goal of this study was to investigate the deleterious effects of α-synuclein in human dopaminergic Lund human mesencephalic (LUHMES) cells. In particular, we investigated a variant of α-synuclein protein targeted to mitochondria, since rapidly evolving concepts suggest a particular role of α-synuclein toxicity at the level of mitochondria in PD. Therefore, we have engineered novel adeno-associated virus type-2 based models for α-synuclein protein expression in the cytosol or in mitochondria. Overexpression of cytosolic and the mitochondrial variants of α-synuclein severely disrupted the dendritic network, induced loss of cellular ATP, enhanced mitochondrial ROS production, and was associated with activation of caspases and dopaminergic cell death in a time-dependent manner. In addition, real-time analysis of mitochondrial bioenergetics using Seahorse Bioscience system following AAV infection elicited a complete damage to mitochondrial respiration capacity in dopaminergic neurons. Mitochondrial targeted expression of α-synuclein appears to be more toxic than the cytosolic form of α-synuclein. In addition, ultrastructural mitochondrial morphological analysis by transmission electron microscopy illustrated a number of deformed cristae in cytosolic form and a complete loss of cristae structure and massively swollen mitochondria after expression of mitochondrial targeted α-synuclein in the human dopaminergic neurons. Furthermore, we addressed the question whether dopaminergic neuronal cell death induced by α-synuclein could be rescued by pharmacological approaches. We found that inhibition of caspases by QVD significantly ameliorated α-synuclein induced dopaminergic neuronal death. Interestingly, inhibition of caspases preserved neuronal network integrity, ATP levels and mitochondrial respiration capacity in both paradigms of cytosolic and mitochondrial α-synuclein overexpression. Overall, our findings show that cytosolic as well as mitochondrial targeted expression of α-synuclein is detrimental to human dopaminergic neurons, and inhibition of caspases amend α-synuclein toxicity. Thus, caspase inhibitors provide promising therapeutic potential to prevent dopaminergic neuronal death in Parkinson's syndromes that are associated with α-synuclein toxicity.

Disclosures: G.K. Ganjam: None. **A.M. Dolga:** None. **K. Bolte:** None. **S. Neitemeier:** None. **M. Höllerhage:** None. **W.E. Oertel:** None. **G.U. Hoeglinger:** None. **C. Culmsee:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.11

Topic: C.03. Parkinson's Disease

Support: Parkinson's UK Grant G-1203

Title: Rab11 modulates alpha-synuclein mediated defects in synaptic transmission and behaviour

Authors: *F. GIORGINI[1], C. BREDA[1], M. L. NUGENT[2], J. G. ESTRANERO[1], C. P. KYRIACOU[1], T. F. OUTEIRO[3], J. R. STEINERT[2];
[1]Dept. of Genet., Univ. of Leicester, Leicester, United Kingdom; [2]MRC Toxicology Unit, Leicester, United Kingdom; [3]Univ. of Goettingen, Goettingen, Germany

Abstract: A central pathological hallmark of Parkinson's disease (PD) is the presence of proteinaceous depositions known as Lewy bodies, which consist largely of the protein alpha-synuclein (aSyn). Mutations, multiplications, and polymorphisms in the gene encoding aSyn are associated with familial forms of PD and susceptibility to idiopathic PD. Alterations in aSyn impair neuronal vesicle formation/transport, and likely contribute to PD pathogenesis by neuronal dysfunction and degeneration. aSyn is functionally associated with several Rab family GTPases, which perform various functions in vesicle trafficking. Here we explore the role of Rab11 - which is critical in endosomal recycling - in the pathogenesis of PD using Drosophila models of aSyn toxicity. We find that aSyn potentiates synaptic transmission at the larval neuromuscular junction by increasing synaptic vesicle size, and that these alterations are reversed by Rab11. Furthermore, Rab11 ameliorates several aSyn-dependent phenotypes in both larvae and adult fruit flies, including locomotor activity, degeneration of dopaminergic neurons, and shortened lifespan. This work highlights the importance of Rab11 in aSyn-dependent defects, particularly in the modulation of synaptic dysfunction due to changes in synaptic vesicle size. Notably, our data suggest that targeting Rab11 activity may have unexplored therapeutic value in PD.

Disclosures: F. Giorgini: None. **C. Breda:** None. **M.L. Nugent:** None. **J.G. Estranero:** None. **C.P. Kyriacou:** None. **T.F. Outeiro:** None. **J.R. Steinert:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.12

Topic: C.03. Parkinson's Disease

Support: EXPL/BIM-MED/0822/2013

SFRH/BD/90365/2012

Title: MicroRNA-124 loaded nanoparticles modulate neurogenesis in the subventricular zone

Authors: *C. M. SARAIVA[1], J. M. PAIVA[2], L. FERREIRA[3,2], L. I. BERNARDINO[4]; [1]Fac. of Hlth. Sci., Hlth. Sci. Res. Ctr. - Univ. of Be, Covilhã, Portugal; [2]Biocant, Ctr. of Innovation in Biotech., Cantanhede, Portugal; [3]Ctr. for Neurosci. and Cell Biol., Coimbra, Portugal; [4]Hlth. Sci. Res. Ctr., Covilhã, Portugal

Abstract: The subventricular zone (SVZ) lining the lateral ventricles comprises the largest population of neural stem cells (NSCs) in the adult mammalian brain. NSCs are multipotent and can give rise to neurons and glia cells. MicroRNA (miR)-124 has been recently described to trigger neuron commitment of NSCs. However, current strategies to deliver miRs into cells or tissues are not efficient. Thus, identifying new platforms to deliver proneurogenic molecules such as miR124 is crucial to boost neurogenesis and to take advantage of the huge potential of endogenous NSCs to repair the damaged brain. The main goal of this work is to study the inductive effect of miR-124-loaded nanoparticles (miR-124 NPs) in the differentiation of NSCs into new neurons. For this purpose, neonatal P1-3 C57BL/6 mice were used to obtain stem/progenitors cell cultures from the SVZ. The cells were grown as neurospheres for 5 days and then seeded on coverslips and allowed to adhere. The resultant cell monolayer was then transfected with several concentrations (1, 10 and 20 μg/mL) of NPs complexed with 200 nM of miR-124. We found that 1 μg/mL of NPs did not interfere with cell toxicity (accessed by propidium iodide and TUNEL assays) or proliferation (BrdU assay). Interestingly, 1μg/mL of NPs complexed with miR-124 was able to increase the differentiation into neurons (NeuN-immunoreactivity) in about 25% compared with non-treated (controls) or void NPs treated cultures. Additionally, no change in the oligodendrocyte commitment was observed (olig2-immunoreactivity). The relative mRNA amount of two validated miR-124 targets, sox9 and jagged1, was also assessed by qPCR. As expected, miR-124 NPs reduced the expression of both genes as compared with controls. Moreover, miR124 NPs induced a significant decrease in the number of sox9-immunoreactive positive cells (approximately 20%) as compared with controls. Taken together, our results showed that the presence of miR-124 delivered by NPs increase the neuronal commitment of SVZ stem/progenitors cells, being the 1μg/mL NPs - 200 nM miR-124 the most suitable formulation. These results provide clear evidences to support the use of miR-124 NPs as a new therapeutic approach to boost brain repair endogenous mechanisms in the setting of neurodegenerative diseases.

Disclosures: **C.M. Saraiva:** None. **J.M. Paiva:** None. **L. Ferreira:** None. **L.I. Bernardino:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.13

Topic: C.03. Parkinson's Disease

Support: GUMC Funds

Title: Towards a novel therapy for synucleinopathies: Candesartan cilexetil inhibits oligomeric alpha-synuclein-induced neuroinflammation

Authors: ***S. G. DANIELE**, K. MAGUIRE-ZEISS;
Neurosci., Georgetown Univ. Med. Ctr., Washington, DC

Abstract: Synucleinopathies, such as Parkinson's disease, are progressive neurodegenerative disorders characterized by the loss of selective neurons and the accumulation of oligomeric α-synuclein in neuronal cell bodies and neurites. Importantly, glial activation is present at both early and late stages of these disorders, indicating a molecular interplay between neuroinflammation and disease progression. We have demonstrated that oligomeric synuclein induces complex morphofunctional changes in primary microglia including a shift to ambeoid morphology, enhanced nuclear translocation of the NFkB p65 subunit, and increased expression of proinflammatory molecules and toll-like receptors. In addition, this activation is mediated through the toll-like receptor adaptor protein, MyD88. Here we demonstrate that oligomeric synuclein activates microglia by directly interacting with the toll-like receptor heterodimer TLR1/2 at the cell's surface. We further show that the FDA-approved angiotensin II receptor blocker, candesartan cilexetil, attenuates synuclein-induced microglial activation. In our paradigm, the anti-inflammatory effects of candesartan are evidenced by a decrease in the biochemical and morphological immunophenotype of activated microglia, devoid of a concurrent increase in molecules indicative of immune-resolution and repair, such as IL-10. Since microglia do not express angiotensin II receptors and oligomeric synuclein directly engages TLR1/2, we hypothesize that candesartan acts antagonistic to the TLR1/2 signaling pathway. Our work supports the further development of therapeutics directed at attenuating the neuroinflammatory response in synucleinopathies.

Disclosures: **S.G. Daniele:** None. **K. Maguire-Zeiss:** None.

Nanosymposium

199. Neuroprotection in Parkinson's Disease: Mechanisms and Therapies

Location: 206

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 199.14

Topic: B.02. Ligand-Gated Ion Channels

Title: Hybrid nanostructures for exclusive inhibition of extrasynaptic NMDA receptors

Authors: E. MOLOKANOVA[1], G. H. BRAUN[2], *A. SAVTCHENKO[3];
[1]Nanotools Biosci., Encinitas, CA; [2]Sanford Burnahm Med. Res. Inst., La Jolla, CA; [3]Univ. of California - San Diego, La Jolla, CA

Abstract: Brain disorders take a heavy economic and social toll on our society. Glutamatergic cytotoxicity mediated by overactivation of NMDA receptors (NMDARs) is implicated in many neurological disorders, including ischemic stroke, brain trauma, amyotrophic lateral sclerosis, Alzheimer's, Parkinson's, and Huntington's diseases. To be therapeutically viable, NMDAR antagonists must block only excessive pathological activation of receptors, while preserving their normal physiological role in synaptic neurotransmission. Here we report a novel NMDAR antagonist that satisfies this two-fold requirement. Given that synaptic NMDARs (sNMDARs) support physiological processes and extrasynaptic NMDARs (eNMDARs) mediate pathological pathways, we decided to design eNMDAR-specific antagonists by exploiting differences in spatial characteristics of subcellular locations of sNMDARs and eNMDARs. Here we present a nanostructure comprising the NMDAR antagonist attached via PEG polymers to a gold (Au) nanoparticle (Au-Memantine). This nanostructure engineered to be larger than the synaptic cleft was capable of efficient and selective inhibition of eNMDARs, while having no effect on sNMDARs and synaptic transmission in cerebrocortical neurons. Furthermore, Au-Memantine was able to prevent dendritic spine loss triggered by Aβ oligomers in organotypic hippocampal slices, and was more effective than free memantine. The ability to manipulate eNMDAR-mediated pathways is crucial both for understanding the mechanisms of neurological disorders and for development of rational approaches for pharmacological treatments. The advantage of proposed nanostructures is that all three components (memantine, gold, and PEG) are approved by FDA for use in humans. Due to its size and remarkable pharmacological properties, Au-Memantine can discriminate between different NMDAR-mediated pathways responsible for normal and pathological brain activities, thus ensuring potentially improved clinically

tolerability. Our results could have far-reaching implications in both basic and translational neuroscience as this study provides proof-of-concept for a new class of neuroprotective drugs for a wide spectrum of neurological disorders with a dichotomic synaptic vs. extrasynaptic activity pattern.

Disclosures: E. Molokanova: None. **G.H. Braun:** None. **A. Savtchenko:** None.

Nanosymposium

200. Fragile X Syndrome: Molecular Mechanisms and Therapeutic Strategies

Location: 147B

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 200.01

Topic: A.04. Stem Cells

Support: NIMH R01 MH084880-05

Lois Pope LIFE Foundation Development Award

Title: Involvement of FMR4, a trans-active noncoding RNA associated with Fragile X, in neural development

Authors: *V. J. PESCHANSKY[1], C. PASTORI[2], Z. ZEIER[2], D. MOTTI[3], J. L. BIXBY[3], V. P. LEMMON[3], J. P. SILVA[2], C. WAHLESTEDT[2];
[2]Ctr. for Therapeut. Innovation, Psychiatry & Behavioral Sci., [3]Miami Project to Cure Paralysis, [1]Univ. of Miami, Miller Sch. of Med., Miami, FL

Abstract: Fragile X syndrome (FXS) is the most common cause of inherited mental retardation, and a leading form of genetically-linked autism. FXS is classically regarded as a single-gene disorder due to silencing of an expanded CGG repeat region in the fragile X mental retardation 1 (*FMR1)* gene. However, there is considerable variability in presentation among patients that thus far, is incompletely understood. Recent work by our laboratory and others has identified several long non-protein coding RNAs (lncRNAs) at the *FMR1* locus, including *FMR4*, a primate specific transcript. As with *FMR1, FMR4* is silenced due to DNA and histone methylation of the fully expanded repeat. There is insubstantial *cis*-regulation of *FMR1* by *FMR4*; thus, we sought *trans*-regulated *FMR4* targets in response to overexpression and knockdown of *FMR4 in vitro*. Gene expression and chromatin IP microarray experiments in HEK293T cells identified differentially expressed *FMR4* responsive genes mapping to canonical pathways including cell cycle regulation, neurogenesis, axon guidance and ion transport. We validated these putative

FMR4 targets with qPCR in HEK293T cells. Using a human neurosphere system, we demonstrated that *FMR4* is active in neural precursor cells (hNPCs) and regulates expression of target genes involved in cell cycling, G-protein receptors and the ubiquitin-proteasome pathway. While *FMR1* mRNA expression does not follow a particular pattern throughout 30 days of *in vitro* differentiation, *FMR4* levels decrease over the same time period. This suggests that *FMR4* may be more active at earlier developmental stages. Colony formation and S-phase marker assays in HEK293T and hNPCs further support a role for this lncRNA in cellular proliferation, and suggest that *FMR4* likely increases cell division. Finally, putative protein partners of *FMR4* were identified by RNA-IP and mass spectrometry using cells expressing an MS2-tagged *FMR4*. These data significantly expand our knowledge of the function of the lncRNA *FMR4*. In combination with the recent discovery of *FMR5* and *FMR6*, two novel lncRNAs expressed from the *FMR1* locus that are also differentially affected by both full mutation and premutation repeat expansions, these data suggest several novel mechanisms that may help to explain the pathogenesis of FXS and associated disorders.

Disclosures: **V.J. Peschansky:** None. **C. Pastori:** None. **Z. Zeier:** None. **D. Motti:** None. **J.L. Bixby:** None. **V.P. Lemmon:** None. **J.P. Silva:** None. **C. Wahlestedt:** None.

Nanosymposium

200. Fragile X Syndrome: Molecular Mechanisms and Therapeutic Strategies

Location: 147B

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 200.02

Topic: C.06. Developmental Disorders

Support: NIH Grant F31MH097451

NIH Grant 1K08NS069809

VA Grant BLRD #1I01BX001689

NIH Grant RO1MH085798

NIH Grant F31NS073372

NIH Grant T32GM008322

National Fragile X Foundation student fellowship

Title: Neuronal dysfunction in Fragile X premutation model mice

Authors: *A. RENOUX[1], A. J. ILIFF[1], K. J. SALA-HAMRICK[1], N. M. CARDUCCI[1], P. K. TODD[2], M. A. SUTTON[3];
[2]Neurol., [3]Mol. and Behavioral Neurosci. Inst., [1]Univ. of Michigan, Ann Arbor, MI

Abstract: Impairments in synaptic plasticity and neuronal excitability are thought to underlie disorders such as Fragile X Syndrome (FXS) and autism. One protein important for the regulation of synaptic plasticity is the RNA-binding Fragile X mental retardation protein (FMRP), which acts to inhibit translation at the synapse. FXS results from large (greater than 200) CGG repeat expansions in the 5' untranslated region (UTR) of the FMR1 gene encoding FMRP; these CGG repeat expansions trigger gene methylation and transcriptional silencing, resulting in the absence of FMRP. In contrast, "premutation" range repeats of 55-200 CGGs result in the age related neurodegenerative disorder Fragile X-associated Tremor Ataxia Syndrome (FXTAS). Premutation sized repeats are associated with increased FMR1 mRNA transcription but impaired FMRP translational efficiency, causing lower basal levels and reduced activity-dependent FMRP synthesis. Recently, human premutation carriers have been shown to have higher rates of autism, hyperactivity, and other psychiatric symptoms, raising the possibility that altered FMRP synthesis contributes to these phenotypes. Using a premutation mouse model which contains 120 repeats knocked-in the murine Fmr1 5'UTR (CGG KI), we have identified alterations in metabotropic glutamate receptor (mGluR)-dependent plasticity. These alterations correlate with impaired dendritic FMRP synthesis in response to mGluR agonists. In addition to defects in synaptic plasticity, we find age-dependent alterations in sensorimotor gating as measured by prepulse inhibition (PPI). These findings suggest that impaired FMRP production may contribute to altered synaptic function and behavioral abnormalities in CGG KI mice and suggest a role for dysregulated FMRP synthesis in cognitive dysfunction.

Disclosures: A. Renoux: None. **A.J. Iliff:** None. **K.J. Sala-Hamrick:** None. **N.M. Carducci:** None. **P.K. Todd:** None. **M.A. Sutton:** None.

Nanosymposium

200. Fragile X Syndrome: Molecular Mechanisms and Therapeutic Strategies

Location: 147B

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 200.03

Topic: C.06. Developmental Disorders

Support: Start-up fund from University of Illinois at Urbana-Champaign

Title: The study of ubiquitin E3 ligase Mdm2 in MEF2- and FMRP-dependent synapse elimination

Authors: *N.-P. TSAI[1], K. HUBER[2];
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Abstract: Fragile X syndrome (FXS) is the most common inherited form of mental retardation and autism, with a population prevalence of about 1/4,000 males and 1/8,000 females. FXS is caused by transcriptional silencing or loss-of-function mutations in the *Fmr1* gene, which encodes for the Fragile X Mental Retardation Protein (FMRP). FMRP is an RNA-binding protein which regulates mRNA transport and translation in dendrites. In FXS patients and the mouse model of FXS, *Fmr1* KO mice, an increased number of dendritic spines, the point of contact of excitatory synapses, are observed. This is consistent with multiple signs of neuronal hyperactivity in FXS, including hypersensitivity to sensory stimuli, anxiety and seizures, suggesting a deficit in maintaining proper synaptic development. Recent study suggested that the synapse elimination triggered by the activity-dependent transcription factor, Myocyte Enhancer Factor 2 (MEF2), is abolished in hippocampal neurons of *Fmr1* KO mice. In the current study, we first identified the ubiquitination and degradation of PSD-95 mediated by an ubiquitin E3 ligase, Murine Double Minute 2 (Mdm2), is the key step toward MEF2-induced synapse elimination. Then we revealed that the improperly distributed Mdm2, which is triggered by imbalanced phosphorylation on Mdm2, is responsible for the absent synapse elimination in *Fmr1* KO neurons. Correcting the phosphorylation event on Mdm2 in *Fmr1* KO neurons rescued MEF2-induced synapse elimination. These data provide valuable information for understanding synapse development during physiological condition as well as pathophysiological conditions, such as neurodevelopmental disorders. Based on the identified molecular mechanism mediated by multiple autism-related genes, the finding from this study may also benefit future research on other autism spectrum disorders.

Disclosures: **N. Tsai:** None. **K. Huber:** None.

Nanosymposium

200. Fragile X Syndrome: Molecular Mechanisms and Therapeutic Strategies

Location: 147B

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 200.04

Topic: C.06. Developmental Disorders

Support: NIH Grant MH093661

Arnold O. Beckman Award

Spastics Paralysis Research Foundation of the Illinois-Eastern Iowa District of Kiwanis International

Dr. Miriam and Sheldon Adelson Medical Research Foundation

Rainwater Charitable Foundation

Title: FMRP associates with MOV10 to modulate AGO2 function in the 3'UTR

Authors: *S. CEMAN[1], P. J. KENNY[1], H. ZHOU[2], M. KIM[3], G. SKARIAH[3], R. KHETANI[4], J. DRNEVICH[4], M. LUZ ARCILA[2], K. S. KOSIK[2];
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Abstract: The fragile X mental retardation protein FMRP is an RNA binding protein that regulates translation of its bound mRNAs through incompletely defined mechanisms. FMRP has been linked to the microRNA pathway and we show here that it is associated with MOV10, a putative helicase that is also associated with the microRNA pathway. We demonstrate that FMRP associates with MOV10 directly in an RNA-dependent manner and facilitates MOV10-association with RNAs in brain. We identified the RNAs recognized by MOV10 using RNA-IP and iCLIP. These RNAs contained a GC-rich secondary structure. MOV10, like FMRP also binds a RNA G-quadruplex in vitro, suggesting that G-quadruplexes are among the GC-rich secondary structures recognized by MOV10. Importantly, FMRP and MOV10 bind a common subset of RNAs. RNAseq of the transcriptomes upon knockdown and overexpression of MOV10 revealed subsets of RNAs that were increased in the absence of MOV10, as would be expected if MOV10 functioned in the microRNA pathway. Significantly, we also identified a subset of RNAs that decreased in the absence of MOV10, suggesting that MOV10 protected those RNAs from degradation. Accordingly, in the absence of MOV10, that same subset of RNAs was found to be more associated with AGO2. Importantly, FMRP binding in close proximity to MOV10 in the 3'UTR, resulted in protection of the mRNAs from degradation. We provide evidence that MOV10 has a dual function in regulating translation: it facilitates microRNA-mediated translation of a subset of RNAs, but also has the novel role of increasing the expression of a different subset of RNAs by preventing AGO2 function through FMRP. In summary, we have identified a new mechanism for FMRP-mediated translational regulation through its association with MOV10.

Disclosures: S. Ceman: None. **P.J. Kenny:** None. **H. Zhou:** None. **M. Kim:** None. **G. Skariah:** None. **R. Khetani:** None. **J. Drnevich:** None. **M. Luz Arcila:** None. **K.S. Kosik:** None.

Nanosymposium

200. Fragile X Syndrome: Molecular Mechanisms and Therapeutic Strategies

Location: 147B

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 200.05

Topic: C.06. Developmental Disorders

Support: NIDA Fellowships T32 DA007290 & F32 DA027265

FRAXA Research Foundation Fellowship

Simons Foundation SFARI Grant

NIDA Grants DA008277, DA027664, DA030590, DA019666, R21 DA033457, K02 DA035459

NINDS Grant NS062158

Eleanor & Miles Shore Harvard Medical School Fellowship

The Jonathan Edward Brooking Mental Health Research Scholar Award

Title: Fragile X mental retardation protein regulates cocaine-induced behavioral and synaptic plasticity

Authors: *L. N. SMITH[1,2], J. P. JEDYNAK[1,5], M. R. FONTENOT[3], C. F. HALE[2], K. C. DIETZ[2], M. TANIGUCHI[1,2], F. S. THOMAS[2], B. C. ZIRLIN[1,2], S. G. BIRNBAUM[2], K. M. HUBER[4], M. J. THOMAS[5,6], C. W. COWAN[1,2];
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Abstract: Chronic exposure to drugs of abuse establishes persistent, maladaptive changes in behavior, as well as in synaptic structure and function that, in notable ways, resemble learning and memory adaptations normally induced by experience. Here we show that the fragile X mental retardation protein (FMRP), an RNA binding protein that promotes synapse weakening and elimination, plays a critical role in regulating behavioral and synaptic plasticities that evolve over multiple psychostimulant drug exposures. Specifically, *Fmr1* knockout (KO) mice show significant reductions in psychomotor sensitization to cocaine and amphetamine, and deletion of the *Fmr1* gene selectively in the adult nucleus accumbens (NAc) recapitulates this defect,

revealing a critical post-developmental role for FMRP in drug-induced adaptation. Consistent with FMRP's known role in synapse weakening and elimination, we found that Fragile X mice have enhanced structural and functional synaptic connectivity (dendritic spine density and mEPSC frequency and amplitude) in NAc shell medium spiny neurons following repeated cocaine administration compared to wild-type littermates. In addition, we observe a strong deficit in cocaine-induced reward (contextual place conditioning) in Fragile X mice, which is rescued by genetically reducing metabotropic glutamate receptor 5 (mGluR5) expression in *Fmr1* KO littermates. Since our Fragile X mice show normal contextual associations with an aversive stimulus and normal natural (food/sucrose) reward behavior, this deficit may be specific to drug-related reward. Interestingly, impaired cocaine reward is not driven by loss of FMRP in the NAc, indicating that FMRP plays separable roles in different drug-induced behaviors in different brain regions. Broadly, our findings reveal a critical role for FMRP, particularly in the ventral striatum, in the behavioral and synaptic responses normally induced by cocaine exposure. In tandem, our observations illustrate how loss of FMRP alters the ability of neurons to make normal synaptic responses to experience, which may offer insight into mechanisms underlying the learning impairments observed in Fragile X.

Disclosures: **L.N. Smith:** None. **J.P. Jedynak:** None. **M.R. Fontenot:** None. **C.F. Hale:** None. **K.C. Dietz:** None. **M. Taniguchi:** None. **F.S. Thomas:** None. **B.C. Zirlin:** None. **S.G. Birnbaum:** None. **K.M. Huber:** None. **M.J. Thomas:** None. **C.W. Cowan:** None.

Nanosymposium

200. Fragile X Syndrome: Molecular Mechanisms and Therapeutic Strategies

Location: 147B

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 200.06

Topic: C.06. Developmental Disorders

Support: NIH grant RO1MH080434

NIH grant RO1MH078972

FRAXA

John Merck Fund

Title: Fragile X proteins regulate neuronal differentiation and maturation

Authors: *X. ZHAO, W. GUO, Y. LI;
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Abstract: Fragile X mental retardation protein (FMRP) and its paralogs FXR1 and FXR2 are neuron-enriched selective RNA-binding protein. Functional loss of FMRP leads to Fragile X syndrome, the most common monogenetic form of inherited intellectual disability and autism, with learning disability. The contributions of FXR1 and FXR2 to human diseases are not as clear. We have found that FMRP and FXR2 regulate neural stem cell differentiation and neuronal maturation, which has significant impact on hippocampus-dependent learning and memory. Our work unveils novel mechanisms regulating neuronal development by Fragile X proteins and provides new insight into the etiology of neurodevelopmental disorders with learning impairment.

Disclosures: X. Zhao: None. **W. Guo:** None. **Y. Li:** None.

Nanosymposium

200. Fragile X Syndrome: Molecular Mechanisms and Therapeutic Strategies

Location: 147B

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 200.07

Topic: C.06. Developmental Disorders

Support: fraxa postdoctoral fellowship

Title: Disrupted mGluR5-Homer scaffolds in a mouse model of fragile X syndrome

Authors: *W. GUO;
Neurosci., UT Southwestern Med. Ctr., Dallas, TX

Abstract: Fragile X Syndrome (FXS) is caused by transcriptional silencing of the Fmr1 gene and a leading genetic cause of autism. Enhanced metabotropic glutamate receptor subunit 5 (mGluR5) function is causally associated with the pathophysiology of Fragile X Syndrome. We recently discovered a molecular basis for mGluR5 dysfunction in Fmr1 KO mice - a decreased association of mGluR5 with the scaffolding protein Homer. In the brains of Fmr1 KO mice there is a reduced interaction of mGluR5 with a scaffolding protein called Homer, which normally keeps mGluR5 inactive and regulates mGluR5 responses to glutamate. Homer binds to the intracellular C-terminal tail of group 1 mGluRs and form multi-protein signaling complexes at the postsynaptic density with mGluRs and their downstream effector. All Homer isoforms share

a common EVH1 domain at the N-terminus, which binds to mGluR1a, mGluR5, PI3 Kinase enhancer (PIKE), IP3 receptor, SHANK and others. In Fmr1 KO mice mGluR5 is less associated with long Homer isoforms and more associated with H1a(Giuffrida, R. et al., 2005, Ronesi et al., 2012). Little is known about how mGluR5-Homer scaffolds are regulated in neurons or how loss of Fmr1 leads to disrupted mGluR5-Homer interactions. Here we find that inhibition of Homer phosphorylation rescues disrupted mGluR5-homer scaffolds, circuit hyperexcitability and enhanced protein synthesis rates in Fmr1 KO mice in cultured neurons and acute slices.

Disclosures: W. Guo: None.

Nanosymposium

200. Fragile X Syndrome: Molecular Mechanisms and Therapeutic Strategies

Location: 147B

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 200.08

Topic: C.06. Developmental Disorders

Support: FRAXA

Autism Science Foundation

Tufts Charlton Grant

Title: Astroglia-mediated pathogenic mechanisms in fxs

Authors: *Y. YANG[1], H. HIGASHIMORI[2];
[1]Neurosci., Tufts Univ. Sch. of Med., Boston, MA; [2]Neurosci., Tufts Univ., Boston, MA

Abstract: Recent studies have implicated potentially significant roles for astrocytes in the pathogenesis of neurodevelopmental disorders. Astrocytes undergo a dramatic maturation process following early differentiation from which typical morphology and important functions are acquired. Alterations of the functional maturation of astrocytes in neurodevelopmental disorders may contribute to the pathogenesis of neurodevelopmental disorders. Fragile X syndrome (FXS) is a developmental intellectual disability which shares many clinical features with autism. Although FMRP is enriched in neurons and FMRP is characterized as an important translational repressor in neurons, selective deletion of FMRP in significant number of cortical and hippocampal neurons showed only limited FXS-related phenotypes. The pathogenic roles of the loss of FMRP in non-neuronal glial cells in FXS remain unknown. Here we found a significant down-regulation of important astroglial glutamate transporter GLT1/EAAT2

expression and reduced glutamate uptake in cortex of fmr1$^{-/-}$ mice during postnatal development and human post-mortem FXS samples. We have further found that the selective loss of astroglial FMRP particularly contributes to the reduced GLT1 expression in mismatched wild type neuron and fmr1$^{-/-}$ astroglia co-cultures. The selective loss of astroglial FMRP alone is sufficient to increase the firing rate of WT neurons in co-cultures. We further generated the inducible astro-fmr1-cKO mice to investigate the pathogenic role of the selective deletion of astroglial FMRP in FXS in vivo. We have analyzed a wide range of synaptic and behavior phenotypes on the astro-fmr1-cKO mice and observed certain FXS-related phenotypes. We will present these results during the nanosyposium.

Disclosures: Y. Yang: None. **H. Higashimori:** None.

Nanosymposium

200. Fragile X Syndrome: Molecular Mechanisms and Therapeutic Strategies

Location: 147B

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 200.09

Topic: C.06. Developmental Disorders

Support: NIH Grant MH090237

Title: Neuronal cell type specific expression of axonal Fragile X granules and associated mRNAs

Authors: *M. R. AKINS, M. E. MITCHELL, L. I. TSAI, G. J. WENK;
Dept of Biol., Drexel Univ., Philadelphia, PA

Abstract: Local protein synthesis plays a crucial role in the formation, maintenance and plasticity of neuronal circuits. Local mRNA translation has been extensively investigated in axon outgrowth during development and in postsynaptic plasticity in mature circuits. More recent evidence has suggested that local translation may also regulate presynaptic function. Notably, Fragile X granules (FXGs) are axonal and presynaptic structures that contain Fragile X proteins, ribosomes, and mRNA, including the messages encoding the proteins β-catenin and OMP (olfactory marker protein). Fragile X granules are expressed within a subset of neuronal circuits, and the developmental pattern of expression differs among these brain regions. We therefore sought to determine whether FXGs are a uniform or diverse population of RNA granules. Here we show that FXGs comprise four distinct granule types based on protein composition. These types display region-selective expression, with homogeneous granule composition in axons from

the same cell type within individual brain regions. This circuit and cell type specificity in axonal localization of FXG components is seen even though all of the known FXG components are expressed broadly, including in neurons in which these components do not localize to axons. To further investigate this cell-type specific regulation, we examined the axonal localization of FXGs and the omp mRNA in the olfactory system. This analysis revealed that olfactory subsystems exhibit differential expression of both FXGs and axonal omp mRNA, despite the observation that all olfactory neuronal types express FXG components as well as OMP. Taken together, these studies show that FXGs comprise a family of messenger ribonucleoprotein particles within axons in the central nervous system that are likely to carry out circuit-specific roles in the regulation of mRNA translation. Further, even closely related neuronal types exhibit differential capacity for axonal protein synthesis, likely reflecting functional differences in these circuits. The identification of multiple subsets of FXGs with distinct mRNA cargos therefore presents a potential mechanism for regulating local axonal translation and its output in a circuit-selective manner.

Disclosures: **M.R. Akins:** None. **M.E. Mitchell:** None. **L.I. Tsai:** None. **G.J. Wenk:** None.

Nanosymposium

200. Fragile X Syndrome: Molecular Mechanisms and Therapeutic Strategies

Location: 147B

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 200.10

Topic: C.06. Developmental Disorders

Support: FRAXA Research Foundation

NIH Grant MH085617

NIH Grant NS045711

NIH Grant HD056370

Title: Multiple strategies for targeted reduction of PI3K signaling rescue molecular, cellular and cognitive phenotypes in animal models of Fragile X syndrome

Authors: ***C. GROSS**[1,4], C.-W. CHANG[5], S. M. KELLY[1,6], N. RAJ[1], W.-R. GUO[5], A. J. WHYTE[1], S. W. DANIELSON[1], M. Q. JIANG[1], C.-B. CHAN[2], K. YE[2], J. R. GIBSON[5], K. H. MOBERG[1], S. L. GOURLEY[3], K. M. HUBER[5], G. J. BASSELL[1];

[1]Cell Biol., [3]Pediatrics, [2]Emory Univ., ATLANTA, GA; [4]Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [5]UT Southwestern, Dallas, TX; [6]The Col. of Wooster, Wooster, OH

Abstract: Dysregulated neuronal signaling and protein synthesis, caused by loss of FMRP, may underlie cognitive impairment in Fragile X syndrome (FXS), a common form of inherited intellectual disability and autism. It remains unclear how FMRP deficiency leads to such broad defects in signal transduction and protein synthesis essential for neuronal function and cognition. We hypothesize that excess PI3K activity due to loss of FMRP-mediated regulation of the PI3K signaling complex in FXS is responsible for defects in activity-regulated protein synthesis crucial for neuronal function. Here, we report that reduction of the PI3K catalytic subunit p110β or the PI3K enhancer PIKE, two confirmed FMRP targets, rescues activity-dependent molecular, cellular and behavioral defects in mouse and Drosophila FXS models. Genetic reduction of p110β or PIKE improves nest building behavior in wild type and Fmr1 KO mice. Moreover, both genetic rescue strategies significantly reduce susceptibility of Fmr1 KO mice to audiogenic seizures. Notably, postnatal reduction of p110β restored higher-order cognitive function in a prefrontal cortex-selective FXS mouse model. Our results provide strong rationale for an essential role of p110β and PIKE in the FXS disease phenotype.

Disclosures: **C. Gross:** None. **C. Chang:** None. **S.M. Kelly:** None. **N. Raj:** None. **W. Guo:** None. **A.J. Whyte:** None. **S.W. Danielson:** None. **M.Q. Jiang:** None. **C. Chan:** None. **K. Ye:** None. **J.R. Gibson:** None. **K.H. Moberg:** None. **S.L. Gourley:** None. **K.M. Huber:** None. **G.J. Bassell:** None.

Nanosymposium

200. Fragile X Syndrome: Molecular Mechanisms and Therapeutic Strategies

Location: 147B

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 200.11

Topic: C.06. Developmental Disorders

Support: FRAXA Foundation

NIH grant MH093445

NIH grant NS034007

NIH grant NS047384

NIH grant NS070526

Title: Testing the therapeutic effects of trifluoperazine on Fragile X syndrome in a mouse model

Authors: *H. WANG[1], F. SETHNA[2], M. ZHANG[1], Q. DING[1], H. KAPHZAN[3], E. KLANN[3], W. FENG[4], Y. FENG[4];
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Abstract: Enhanced protein synthesis and over-activation of Gq-coupled glutamate and acetylcholine receptors have been considered as potential mechanisms underlying the pathophysiology of Fragile X syndrome (FXS). Here, we found that inhibition of calmodulin (CaM) activity by W13 blocked ERK1/2 phosphorylation and the up-regulation of Arc translation triggered by the activation of metabotropic glutamate receptors 1 and 5 (mGluR1/5). W13 also blocked mGluR1/5-mediated synaptic long-term depression (LTD) in hippocampal slices and anaesthetized animals. We further evaluated the therapeutic effects of a known CaM inhibitor trifluroperazine, which can be systemically administrated and has been clinically used in treating psychotic conditions. Intraperitoneal injection of trifluoperazine attenuated audiogenic seizures and corrected multiple FXS-related symptoms including hyperactivity, repetitive behavior, and cognitive impairment in a mouse model of FXS. At the cellular level, trifluroperazine blocked ERK1/2 phosphorylation triggered by the activation of both mGluR1/5 and muscarinic cholinergic receptors. Trifluroperazine also normalized the enhanced protein synthesis in FXS neurons to the wild type level. Our study suggests a potential new application of trifluoperazine in FXS treatment.

Disclosures: H. Wang: None. **F. Sethna:** None. **M. Zhang:** None. **Q. Ding:** None. **H. Kaphzan:** None. **E. Klann:** None. **W. Feng:** None. **Y. Feng:** None.

Nanosymposium

200. Fragile X Syndrome: Molecular Mechanisms and Therapeutic Strategies

Location: 147B

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 200.12

Topic: C.06. Developmental Disorders

Support: Brain and Behavior Research Foundation, NARSAD Young Investigator (P0054266)

Title: Reversing the behavioral phenotypes in fmr1 KO by the reduction of potassium channel, Kv4.2

Authors: *H. LEE, L. JAN;
Physiology, Univ. of California, San Francisco, San Francisco, CA

Abstract: Fragile X syndrome (FXS) is a common form of mental disability and one of the known causes of autism. The mutation responsible for FXS is a large expansion of the trinucleotide CGG repeats that leads to DNA methylation of the fragile X mental retardation gene 1 (FMR1) and transcriptional silencing, resulting in the absence of fragile X mental retardation protein (FMRP), an mRNA binding protein. Although it is widely known that FMRP is critical for metabotropic glutamate receptor (mGluR)-dependent long-term depression (LTD), which has provided a general theme for developing pharmacological drugs for FXS, specific downstream targets of FMRP may also be of therapeutic value. We reported dendritic localization of mRNA of Kv4.2 voltage-gated potassium channel, which regulates synaptic plasticity, and its local translational regulation by FMRP. FMRP suppression of Kv4.2 is revealed by elevation of Kv4.2 in neurons from fmr1 KO mice. Moreover, treating hippocampal slices from fmr1 KO mice with Kv4 channel blocker restores long-term potentiation (LTP) induced by moderate stimuli. To test the effect of Kv4.2 levels in FXS, we generated fmr1 mutant mice with a 50 % reduction in Kv4.2 expression and studied a range of phenotypes with relevance to the human disorders. We found that Kv4.2 reduction in fmr1 KO mice reverses the altered repetitive and perseverative phenotype of fmr1 KO mice. We also found Kv4.2 reduction rescues the deficit in social behaviors. Our results demonstrate that Kv4.2 contributes significantly to the pathogenesis of the disease, a finding that has significant therapeutic implications for FXS.

Disclosures: H. Lee: None. **L. Jan:** None.

Nanosymposium

200. Fragile X Syndrome: Molecular Mechanisms and Therapeutic Strategies

Location: 147B

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 200.13

Topic: C.06. Developmental Disorders

Support: NIH grant 1K99NS087112-01

NIH grant NS034007

NIH grant NS047384

Department of Defense CDMRP award W81XWH-11-1-0389

Title: Modulation of eIF4E restores the balance between protein synthesis and actin dynamics in Fragile X Syndrome

Authors: *E. SANTINI[1], T. HUYNH[2], S. KOO[3], E. PASCIUTO[4], C. BAGNI[5], E. KLANN[3]; [1]Ctr. for Neural Sci., New York Univ., New York, NY; [2]New York Univ., new York, NY; [3]New York Univ., New York, NY; [4]Catholic Univ. of Leuven, Leuven, Belgium; [5]Catholic Univ. of Leuven, Catholic University of, Belgium

Abstract: Fragile X syndrome (FXS), the most frequent form of inherited intellectual disability and autism, is caused by transcriptional silencing of the Fmr1 gene. Genetic deletion of Fmr1 gene (Fmr1 KO) in mice results in a series of synaptic and structural phenotypes in the hippocampus, such as enhanced protein synthesis-dependent long-term depression (LTD; Huber et al., 2002) and increased dendritic spine density (Dölen et al., 2007) caused by altered protein synthesis (Quin et al., 2005) and defective synaptic actin dynamics. We recently demonstrated that pharmacological inhibition of eIF4E/ eIF4G association and consequently protein synthesis, with 4EGI-1 is effective in normalizing behavioral and synaptic autism-like alterations in eIF4E transgenic mice (Santini et al., 2013). It was also shown that genetic and pharmacologic inhibition of PAK signaling pathway, which regulates actin dynamics, normalizes phenotypes in Fmr1 KO mice (Hayashi et al., 2007; Dolan et al., 2013). Finally, it has been demonstrated that activated Rac, which is an upstream regulator of PAK, changes the equilibrium between the cytoplasmic FMRP-interacting protein 1 (CYFIP1) and its association with either eIF4E or Wave regulatory complex (WRC) and thereby, contributes to both regulation of protein synthesis and actin dynamics (De Rubeis et al., 2013). Here we show that 4EGI-1 is efficient in preventing the multiple hippocampal phenotypes observed in Fmr1 KO mice (e.g. normalization of enhanced mGluR-LTD). Interestingly, we found that these effects are protein synthesis-independent in the Fmr1 KO mice. Indeed, 4EGI-1, by interfering with the association of eIF4E with eIF4G perturbs the equilibrium between CYFIP1 interacting with activated Rac/ WRC and CYFIP1 associated to eIF4E. Thus, restoring normal activity of the Rac/ PAK pathway in the Fmr1 KO mice. Overall, our findings suggest that an alternative therapeutic strategy to treat FXS is to restore the balance between protein synthesis and actin dynamics via modulation of eIF4E.

Disclosures: E. Santini: None. **T. Huynh:** None. **S. Koo:** None. **E. Pasciuto:** None. **C. Bagni:** None. **E. Klann:** None.

Nanosymposium

201. Epilepsy: Man and Mouse

Location: 146C

Time: Sunday, November 16, 2014, 1:00 PM - 3:15 PM

Presentation Number: 201.01

Topic: C.07. Epilepsy

Support: NIH HHSN 271201100029C

1-R01-NS065434

Title: Treatment of acute behavioral seizures in the Theiler's murine encephalomyelitis virus model of acquired epilepsy disrupts long-term, but not acute, histopathology

Authors: *M. L. BARKER-HALISKI[1], T. D. HECK[1], E. DAHLE[2], T. H. PRUESS[1], K. S. WILCOX[1,2,3], H. WHITE[1,2,3];
[1]ADD Program, [2]Dept. of Pharmacol. & Toxicology, [3]Interdepartmental Program in Neurosci., Univ. of Utah, Salt Lake City, UT

Abstract: Inflammation represents a significant risk factor for seizure induction and maintenance, with pro-inflammatory cytokines being highly expressed in various animal seizure models and human patients with epilepsy. Infections of the CNS can contribute to the development of chronic epilepsy due to an increased risk of seizures and status epilepticus. Theiler's murine encephalomyelitis virus (TMEV), when injected into brains of C57/Bl6 mice, provides a novel model of infection-induced epilepsy. Approximately 50-65% of infected mice develop acute, handling-induced seizures during the infection, significant CNS inflammation, acute and long-term neuropathology, and mice then develop spontaneous, recurrent seizures weeks later. It is unknown whether treatment during the acute infection can attenuate handling-induced seizures, as well as reduce associated acute and long-term neuropathology. This study investigated the efficacy of the antiseizure drugs (ASDs) valproic acid (VPA) and carbamazepine (CBZ), and the anti-inflammatory antibiotic, minocycline (MIN) on acute TMEV-induced seizures and neuropathology. On Day 0, male C57/Bl6 mice were infected with TMEV. TMEV-infected mice then received VPA (n = 28; 200 mg/kg b.i.d., i.p.), CBZ (n = 28; 20 mg/kg b.i.d., i.p.), MIN (n = 28; 50 mg/kg q.d., i.p.), or vehicle (n = 28) during the TMEV infection period (Day 0-8). Mice were assessed twice daily for 7 days for handling-induced seizures by an experimenter blinded to treatment. Relative to controls, significantly more CBZ-treated mice presented with seizures; VPA and MIN conferred no change in percentage of mice displaying seizures. In mice displaying seizures, VPA, but not CBZ or MIN, reduced the acute seizure burden. Both acute (Day 9) and long-term (Day 36) neurodegeneration and reactive astrogliosis were then analyzed by immunohistochemistry. Relative to brains from vehicle-treated control mice, no treatment prevented the acute (Day 9) decrease in NeuN (neurons) expression or increase in GFAP (astrocytes) expression. Conversely, MIN treatment during the acute seizure period significantly reduced long-term astrogliosis, but was without effect on NeuN immunoreactivity. CBZ treatment did not significantly alter long-term GFAP nor NeuN

immunoreactivity. These data suggest that while treatment with prototype ASDs may alter acute seizure expression and seizure burden in the TMEV model, treatment with anti-inflammatory agents during the acute infection period can attenuate long-term reactive gliosis in this acquired epilepsy model. Such information supports a growing body of evidence suggesting a role for inflammation in seizure disorders.

Disclosures: **M.L. Barker-Haliski:** None. **T.D. Heck:** None. **E. Dahle:** None. **T.H. Pruess:** None. **K.S. Wilcox:** None. **H. White:** None.

Nanosymposium

201. Epilepsy: Man and Mouse

Location: 146C

Time: Sunday, November 16, 2014, 1:00 PM - 3:15 PM

Presentation Number: 201.02

Topic: C.07. Epilepsy

Support: Multiple grants from funding agencies in Asia, Australia, Europe, and North America.

Title: Large scale meta-analysis of genome wide association data in common forms of human epilepsy

Authors: *R. J. BUONO[1,2];
[1]Biomed. Sci., Cooper Med. Sch. of Rowan Uniersity, Camden, NJ; [2]On Behalf of the Intl. League Against Epilepsy, West Hartford, CT

Abstract: Genome wide association was performed to identify genetic susceptibility factors for epilepsy. The distribution of SNP markers across the genome was compared between patients with common forms of epilepsy (N=8696) and control individuals (N=26,157). DNA samples were collected and analyzed by research groups in Asia, Australia, Europe and North America who agreed to share data in order to increase power for a worldwide gene discovery effort. Individual data sets were combined to compare all epilepsy patients with all controls. In addition, patients were divided such that all cases of generalized epilepsy were compared with controls and independently, all cases of focal epilepsy were compared with controls. Genome wide significance was set at p<1.66x10-8 reflecting an empirical Bonferroni correction of 5x10-8 for three separate tests. When comparing all cases to all controls, genome-wide evidence for association was documented for markers within the SCN1A gene at 2q24.3 (strongest variant rs6732655, p=8.71x10-8) and PCDH7 gene at 4p15.1 (rs28498976 p=5.44x10-9). A significant association signal was also found at 2p16.1 (rs2947349 p=9.99x10-9), possibly implicating the

VEK2 or FANCL genes in common forms of epilepsy. Several additional loci reach levels of suggestive association ($p<5x10-7$) and warrant further study as putative positive signals. This first meta-genome-wide association study in epilepsy identifies new loci for common forms of the disease and opens new avenues of research into epileptogenesis and anti-epilepsy therapies. The finding that individual loci can have pleiotropic or specific epilepsy phenotype effects suggests future genetic analysis will benefit from both "lumping" and "splitting" clinical subgroups of epilepsy patients or any epilepsy-related phenotype under study. This work exemplifies the benefits of international cooperation and pooling of resources to increase power to identify the genetic influences on human epilepsy.

Disclosures: R.J. Buono: None.

Nanosymposium

201. Epilepsy: Man and Mouse

Location: 146C

Time: Sunday, November 16, 2014, 1:00 PM - 3:15 PM

Presentation Number: 201.03

Topic: C.07. Epilepsy

Support: NIH Grant NS039587 (MP)

NIH Grant R21NS072099S1 (MP)

NIH Grant U01NS083422 (MP)

Title: Pharmacological scavenging of reactive oxygen species improves cognitive function in an experimental model of Temporal Lobe Epilepsy

Authors: *J. PEARSON[1], L. LIANG[1], B. DAY[2], M. PATEL[1];
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Abstract: Cognitive dysfunction is an important comorbidity of temporal lobe epilepsy (TLE). However, the mechanisms underlying cognitive impairment, specifically deficits in learning and memory associated with TLE remain unclear. We hypothesize that oxidative stress and consequent neuronal loss contributes to cognitive decline associated with injury-induced epileptogenesis. Using a synthetic catalytic antioxidant, we determined if pharmacological scavenging of reactive oxygen species (ROS), 1) prevents seizure-induced neuronal death, and 2) attenuates cognitive dysfunction in a rat model of TLE. Adult male Sprague-Dawley rats were treated with pilocarpine to induce status epilepticus (SE) or saline in control groups. All rats

were injected with scopolamine (1mg/kg) 30 minutes prior to pilocarpine to limit peripheral cholinergic effects and diazepam (10mg/kg) 90 minutes after pilocarpine to terminate SE. Animals received saline or AEOL10150 (5mg/kg) starting 1 hour after pilocarpine and maintained on a q4 dosing schedule for 48 hours. At the 48 hour time point, treatment with AEOL10150 significantly reduced 1) the delayed mortality associated with pilocarpine-induced SE without interfering with SE 2) indices of oxidative stress including glutathione depletion and 3-nitrotyrosine accumulation as detected by HPLC and 3) neuronal death in various regions of the rat brain as indicated by Fluoro-Jade B labeling of degenerating neurons Given that AEOL10150 was neuroprotective, we determined its effects on learning and memory. Specifically, animals were treated as above followed by a gradual tapering of doses over 3 days totaling 15 injections. Rats were then tested for indices of learning and memory in a novel object recognition task (NOR) one week after SE. The NOR task is uniquely suited for testing pilocarpine treated animals at this time point as it is minimally stressful and requires little training of the animal. Pilocarpine-treated animals that received AEOL10150 performed significantly better than saline-treated animals at a level equivalent to controls. These data suggest that pharmacological scavenging of ROS with the catalytic antioxidant is neuroprotective and disease-modifying against cognitive dysfunction in the pilocarpine rat model of experimental TLE.

Disclosures: **J. Pearson:** None. **L. Liang:** None. **B. Day:** 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.; Dr. Day is a consultant for and holds equity in Aeolus Pharmaceutials that is developing metalloporphyrins as potential therapeutic agents.. **M. Patel:** None.

Nanosymposium

201. Epilepsy: Man and Mouse

Location: 146C

Time: Sunday, November 16, 2014, 1:00 PM - 3:15 PM

Presentation Number: 201.04

Topic: C.07. Epilepsy

Support: NIH Grant R01AG032383, R01NS076775, R01NS038572, and K02AG041815 to J.H.

NIH Grant R01NS081203 to J.H. and H.E.S.

NIH Grant R01DA016765 and K02DA023555 to A.J.E.

NASA Grant NX12AB55G to A.J.E.

American Heart Association Training Grant 5T32HL007360-34 to Z.L

NIH Pre-Doctoral Training Grant 5T32GM083831-05 to R.B.

The UTSW EEG Core facility is supported by the Haggerty Center for Brain Injury and Repair.

Title: Aberrant hippocampal neurogenesis drives epilepsy and associated cognitive decline

Authors: *K. CHO[1,2], N. ITO[3], Z. LYBRAND[1], R. BRULET[1], L. ZHANG[1], L. GOOD[1], K. URE[4], S. KERNIE[5], S. BIRNBAUM[1], H. SCHARFMAN[6], A. EISCH[1], J. HSIEH[1];
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Abstract: Epileptic seizures trigger aberrant hippocampal neurogenesis. However, the functional role of adult-generated neurons in the development of chronic epilepsy or its associated cognitive deficits remains to be determined. We found that ablation of adult neurogenesis prior to acute seizures reduced the frequency of recurrent seizures and normalized epilepsy-associated cognitive deficits. Ablation of neurogenesis alleviated seizure-induced aberrant hippocampal neurogenesis, in particular, the production of ectopic granule cells. Furthermore, we identified the basic helix-loop-helix (bHLH) proneural transcription factor NeuroD to be essential for seizure-induced aberrant hippocampal neurogenesis. These findings highlight a causal role of neurogenesis in the generation of chronic epilepsy and suggest that strategies designed to block adult hippocampal neurogenesis, specifically, NeuroD-expressing cells, may have therapeutic potential for reducing spontaneous seizure formation. Our studies also provide a cautionary note regarding neuroregenerative approaches whereby addition of aberrant neurogenesis may exacerbate rather than mitigate disease symptoms.

Disclosures: K. Cho: None. **N. Ito:** None. **Z. Lybrand:** None. **R. Brulet:** None. **L. Zhang:** None. **L. Good:** None. **K. Ure:** None. **S. Kernie:** None. **S. Birnbaum:** None. **H. Scharfman:** None. **A. Eisch:** None. **J. Hsieh:** None.

Nanosymposium

201. Epilepsy: Man and Mouse

Location: 146C

Time: Sunday, November 16, 2014, 1:00 PM - 3:15 PM

Presentation Number: 201.05

Topic: C.07. Epilepsy

Title: Microelectrode array neuronal recordings from intact larval zebrafish

Authors: *M. MEYER, A. PODURI, A. ROTENBERG;
Boston Children's Hosp., Boston, MA

Abstract: The zebra fish model is an emerging tool in experimental epilepsy. Zebrafish larvae, in particular, are advantageous because they can be easily genetically altered and can be used for developmental and drug studies, since agents applied to the bath, penetrate the organism easily. Yet, methods to perform noninvasive brain electrophysiologic recordings in zebrafish are limited. Here, we present a novel method, using multielectrode array recordings, to simultaneously record electrical activity from up to 61 cranial locations of an intact larval zebrafish without penetrating to the intracranial space. The method enables recording of single unit activity and EEG signal. We recorded spontaneous brain single unit activity from zebrafish larva (n=5) by placing the dorsal side of each larva-head onto a microelectrode array (MED64 system) during continuous flow of oxygenated artificial cerebrospinal fluid (aCSF). Recording of spontaneous activity was possible for at least three hours. Spike pattern (continuous or bursting) and frequency at individual electrode locations were reliably recorded. Following 25 mM KCl infusion into the aCSF, spike frequency increased significantly in four of five fish, and burst frequency increased in all. In summary, we recorded single unit activity in vivo from multiple extracranial electrodes positioned on intact larval zebrafish for several hours and documented a seizure-type pattern following exposure to high-concentration of KCl. This high spatial and temporal resolution method complements present zebrafish neurophysilogic techniques and can enable future pharmacological and genetic experiments with this model.

Disclosures: **M. Meyer:** None. **A. Rotenberg:** None. **A. Poduri:** None.

Nanosymposium

201. Epilepsy: Man and Mouse

Location: 146C

Time: Sunday, November 16, 2014, 1:00 PM - 3:15 PM

Presentation Number: 201.06

Topic: C.07. Epilepsy

Support: CIHR Grant 119553

Title: Age-dependent plasticity of cortical GABAergic innervation lessens seizure severity in Cacna1a mutant mice

Authors: E. SAMAROVA[1,2], A. LUPIEN-MEILLEUR[1,2], X. JIANG[1,2], A. M. J. M. VAN DEN MAAGDENBERG[3], J.-C. LACAILLE[2], *E. ROSSIGNOL[1,2];
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Abstract: *CACNA1A* loss-of-function mutations result in cerebellar ataxia and epilepsy in humans. We recently showed that the pre-natal deletion of *Cacna1a* in the $Nkx2.1^{Cre}$; $Cacna1a^{c/c}$ mutant mice, causing the ablation of voltage-gated $Ca_V2.1$ Ca^{2+} channels in forebrain GABAergic interneurons (IN), results in synaptic impairment of parvalbumin (PV) fast-spiking basket cells and induces generalized epilepsy. As *CACNA1A* mutation-associated epilepsy improves with age in patients, we propose that specific compensatory mechanisms may occur that, in the face of cortical disinhibition, re-establish the inhibition/excitation balance with time. We generated conditional mutant mice carrying a post-natal deletion of *Cacna1a* in PV^+ neuronal populations (PV^{Cre};$Cacna1a^{c/c}$). PV^{Cre};$Cacna1a^{c/c}$ mutant mice develop cerebellar ataxia and a mild epileptic phenotype with spike-wave seizures after postnatal day 45 (P45). Surprisingly, these mutants display a two-fold increase in the frequency of miniature inhibitory synaptic currents (mIPSC) in cortical pyramidal cells (PC) at P60. In contrast, the pre-natal $Nkx2.1^{Cre}$; $Cacna1a^{c/c}$ mutants display a significant decrease of mIPSC frequency in cortical PC at P20, suggesting that age-dependent compensatory plasticity changes in GABAergic circuits occur in the PV^{Cre};$Cacna1a^{c/c}$ mutant mice. We show a comparable reduction of GABAergic perisomatic boutons on cortical PC and a similar impairment of synaptic release from PV-INs in both pre-natal ($Nkx2.1^{Cre}$) and post-natal (PV^{Cre}) mutants. Comparatively, we demonstrate a significant increase of functional dendrite-targeting GABAergic projections from somatostatin (SOM) INs in PV^{Cre};$Cacna1a^{c/c}$ mutants. Therefore, we propose that in the face of altered PV-INs synaptic function, progressive reorganization of dendritic inhibition by SOM-INs restricts cortical excitability and lessens seizure severity in PV^{Cre}; $Cacna1a^{c/c}$ mutants. A similar phenomenon has recently been described in chronic post-status epilepticus epilepsy models suggesting that this is a common phenomenon in genetic and non-genetic forms of chronic epilepsy.

Disclosures: E. Samarova: None. **A. Lupien-Meilleur:** None. **X. Jiang:** None. **A.M.J.M. van den Maagdenberg:** None. **J. Lacaille:** None. **E. Rossignol:** None.

Nanosymposium

201. Epilepsy: Man and Mouse

Location: 146C

Time: Sunday, November 16, 2014, 1:00 PM - 3:15 PM

Presentation Number: 201.07

Topic: B.09. Network Interactions

Title: Cortico-cortical relationship in the intracranial EEG second spectrum

Authors: *R. B. JOSHI[1], N. GASPARD[1], I. I. GONCHAROVA[1], R. B. DUCKROW[1], D. DUNCAN[3], J. L. GERRARD[2], D. D. SPENCER[2], L. J. HIRSCH[1], H. P. ZAVERI[1]; [1]Neurol., [2]Neurosurg., Yale Univ., New Haven, CT; [3]Mathematics, Univ. of California - Davis, Davis, CA

Abstract: Objective: A number of networks have been described based on correlations in the fMRI measured BOLD signal, but the electrophysiological bases of these networks are not yet understood. It has been shown previously that the intracranial EEG (icEEG) relationship within the default mode network (DMN) was not significantly greater than relationships between DMN locations and a control location, indicating a lack of support for the fMRI defined DMN in the icEEG (Duncan et al., 2013). However, it has been suggested that low-frequency changes in blood flow may be reflected in correlated low-frequency activity in the band-power time series, the so-called second spectrum, rather than in synchronization of the icEEG. We analyzed icEEG second spectrum relationships between different parts of the brain and examined the second spectrum for evidence of support for the DMN. Methods: We selected one-hour icEEG epochs of background activity for 9 patients with localization related epilepsy. For each one-hour segment, we calculated the running power, at a one-second resolution, in different frequency bands (delta, theta, alpha, beta, and gamma). Magnitude-squared coherence (MSC) below 0.15 Hz was estimated for each band power time-series for every possible electrode contact pair. We aggregated these estimates for all patients and examined how these second spectrum relationships vary with distance, frequency, and lobe. Further, we isolated two test areas within the DMN (anterior cingulate and mesial prefrontal, and posterior cingulate and mesial parietal) and one control area outside the DMN (superior temporal and/or lateral frontal). We tested if the relationship between DMN areas was stronger than the relationship between each of these areas and the control location, as well as all intrahemispheric contact pairs with similar intercontact distance. Results: We observed very low values of second spectrum relationship between different parts of the brain, except at very short distances. We further found that these relationships are strongest in the delta band and decrease with increasing frequency, with the weakest relationships in the gamma band. Our DMN-specific analysis showed no enhanced connectivity in the second spectrum in DMN locations in any frequency band. Conclusions: Though we observed significantly nonzero relationships in lower frequency bands, second spectrum relationships are consistently very low across the entire brain in every frequency band. Our results also suggest a lack of support for the DMN in the icEEG second spectrum.

Disclosures: **R.B. Joshi:** None. **N. Gaspard:** None. **I.I. Goncharova:** None. **R.B. Duckrow:** None. **D. Duncan:** None. **J.L. Gerrard:** None. **D.D. Spencer:** None. **L.J. Hirsch:** None. **H.P. Zaveri:** None.

Nanosymposium

201. Epilepsy: Man and Mouse

Location: 146C

Time: Sunday, November 16, 2014, 1:00 PM - 3:15 PM

Presentation Number: 201.08

Topic: C.07. Epilepsy

Support: CURE

Nebraska LB692

EFA

Title: Brain PPARgamma contributes to the anti-seizure efficacy of the ketogenic diet

Authors: ***T. A. SIMEONE**, K. A. SIMEONE, S. MATTHEWS, K. SAMSON;
Pharmacol., Creighton Univ., Omaha, NE

Abstract: The high fat, low carbohydrate/protein ketogenic diet (KD) is an effective anti-seizure therapy for a broad spectrum of pediatric and adult epilepsies; although, due to the stringency of the diet, it is primarily used in pediatric patients refractory to current anti-seizure medications. The mechanism of KD anti-seizure efficacy is unclear, but it is known that the KD engages anti-inflammatory and anti-oxidant pathways and promotes mitochondrial health. Many of these effects mirror the downstream effects of the nutritionally-regulated transcription factor peroxisome proliferator activated receptor gamma, PPARγ. Here, we tested the hypothesis that PPARγ contributes to the anti-seizure efficacy of the KD. We treated wild-type (WT) and epileptic Kv1.1 knockout (KO) mice with either standard chow or KD and measured nuclear PPARγ protein in brain homogenates with western blots. We found that the KD differentially affected PPARγ isoform expression. The KD increased nuclear PPARγ2 in both WT and Kv1.1KO mice. In WT mice, PPARγ1 was predominant over PPARγ2 (γ2:γ1 ratio ~0.6), whereas in WT+KD mice, γ2 doubled and the two isoforms were expressed equally. Results in the epileptic brains were significantly different from WT: in Kv1.1KO mice, PPARγ2 was predominant and the γ2:γ1 ratio was three-fold greater than WT, which further increased to six fold in Kv1.1KO+KD mice. Co-administration of a PPARγ antagonist, GW9662, prevented KD-

mediated changes in nuclear PPARγ2: γ1 ratios in both WT and Kv1.1KO mice and prevented the anti-seizure efficacy of the KD as measured with video-EEG recordings. We did not detect any seizures in WT mice given GW9662 and GW9662 did not worsen Kv1.1KO seizures. We found that the KD prolongs the latency to flurothyl-induced seizure in WT mice. Therefore, we further tested PPARγ involvement in the KD with PPARγ2KO and neuronal-specific PPARγKO (NKO) mice. KD-mediated increases in flurothyl seizure latencies were lost in PPARγ2KO mice and NKO mice. Further, administration of a PPARγ agonist, pioglitazone, to Kv1.1KO mice mimicked the KD, inducing a similar increase in γ2:γ1 ratio and reducing seizures. Moreover, WT mice given PIO exhibited increased flurothyl seizure latencies. Collectively, we provide pharmacologic and genetic evidence that brain PPARγ is involved in the anti-seizure efficacy afforded by the KD in chronic epilepsy and acute seizures. Our results strongly support the investigation of PPARg[[Unsupported Character - Symbol Font ]]as a therapeutic target for severe, refractory epilepsy.

Disclosures: T.A. Simeone: None. **K.A. Simeone:** None. **S. Matthews:** None. **K. Samson:** None.

Nanosymposium

201. Epilepsy: Man and Mouse

Location: 146C

Time: Sunday, November 16, 2014, 1:00 PM - 3:15 PM

Presentation Number: 201.09

Topic: C.07. Epilepsy

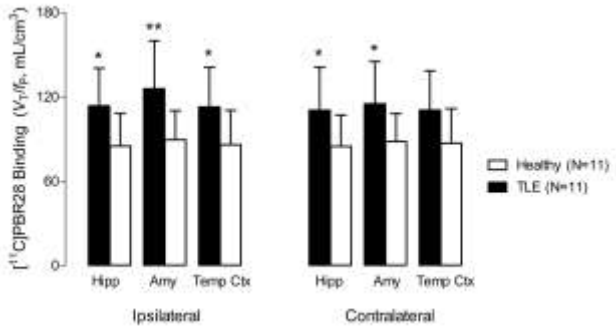
Support: NIH Intramural Program

Title: PET imaging of translocator protein, a neuroinflammatory biomarker, *in vivo* in patients with temporal lobe epilepsy

Authors: L. P. DICKSTEIN[1], P. ZANOTTI-FREGONARA[2], I. DUSTIN[3], J. HONG[2], J.-S. LIOW[1], V. W. PIKE[2], S. S. ZOGHBI[2], *R. B. INNIS[4], W. H. THEODORE[3];
[1]Mol. Imaging Br., NIH, NIMH, BETHESDA, MD; [2]Mol. Imaging Br., NIH, NIMH, Bethesda, MD; [3]Clin. Epilepsy Section, NIH, NINDS, Bethesda, MD; [4]Chief, Molec Imag Br., NIH, NIMH, MIB, BETHESDA, MD

Abstract: **Objective** Translocator protein 18 (TSPO), a biomarker for neuroinflammation, is overexpressed on activated microglia and reactive astrocytes. TSPO can be imaged *in vivo* using the PET ligand [^{11}C]PBR28. We previously showed that [^{11}C]PBR28 uptake is higher ipsilateral

than contralateral to seizure foci in TLE patients. It is unknown whether inflammation is restricted to the area of the seizure focus or is also present in the contralateral hemisphere. To answer this question, we compared regional [^{11}C]PBR28 binding in each hemisphere between TLE patients and healthy controls. **Methods** We scanned 11 TLE patients and 11 controls with [^{11}C]PBR28 and manually sampled arterial blood. Six brain regions were delineated using freesurfer and T1-weighted MRI: hippocampus, amygdala, temporal cortex, temporal pole, fusiform gyrus, and entorrhinal cortex/parahippocampal gyrus. Using a two-tissue compartment model, we quantified [^{11}C]PBR28 binding as distribution volume (V_T) corrected for ligand free fraction (f_P). **Results** In the ipsilateral hemisphere, TLE patients had higher [^{11}C]PBR28 binding than controls in all 6 temporal regions (all $p < 0.05$; see fig). In the contralateral hemisphere, similar increases were seen in the hippocampus, amygdala, and temporal pole (all $p < 0.05$). Marginally significant increases were seen in the contralateral fusiform gyrus, temporal cortex, and entorhinal cortex/parahippocampal gyrus (all $p < 0.06$). **Conclusions** In this study, we show that TSPO is increased both ipsilateral and contralateral to seizure foci relative to that in controls. These findings suggest that both local and widespread neuroinflammation may be at play in epilepsy. TSPO PET imaging could serve as a biomarker to evaluate putative therapeutic effects of anti-inflammatory drug therapy. *Figure legend*: Higher [^{11}C]PBR28 binding (V_T/f_P) in TLE patients than controls ipsilateral and contralateral to seizure foci; Columns are mean and error bars SD; * uncorrected $p<0.05$, ** $p<0.01$ vs. controls by independent samples t-test. Hipp: Hippocampus, Amy: Amygdala, Temp Ctx: Temporal cortex



Disclosures: **L.P. Dickstein:** None. **P. Zanotti-Fregonara:** None. **I. Dustin:** None. **J. Hong:** None. **J. Liow:** None. **V.W. Pike:** None. **S.S. Zoghbi:** None. **R.B. Innis:** None. **W.H. Theodore:** None.

Nanosymposium

202. Traumatic Brain Injury: Animal and Human Studies

Location: 152A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 202.01

Topic: C.10. Trauma

Title: The psychiatric sequelae of concussion

Authors: *M. D. MCCRADY[1], S. RASMUSSEN[2], P. I. ROSEBUSH[3], M. F. MAZUREK[1]; [1]Neurol., [2]McMaster Univ., Hamilton, ON, Canada; [3]Psychiatry, St. Joseph's Hosp., Hamilton, ON, Canada

Abstract: Background: While psychiatric sequelae are known to occur following moderate and severe traumatic brain injury (TBI), these are less well characterized with concussion, or "mild TBI." Methods: We screened 189 cases referred to our neurology service with postconcussion syndrome (PCS). Of these, 59 patients (31%: 32M 27F, mean age=18yrs, range 8-44) had newly presenting clinically significant psychiatric symptomatology, while the remaining 130 (69%) did not. All patients were followed until they were deemed recovered from concussion. The impact of psychiatric symptoms on functioning (academic, personal, social, vocational) was retrospectively assessed with the Global Assessment of Functioning (GAF) scale. Results: The concussions were the result of sports injury (75%), motor vehicle accident (10%), accident/fall (14%) or assault (1%). Six percent of individuals reported a family history of psychiatric disturbances. At the onset of psychiatric symptoms, the mean GAF was 66, indicating moderate difficulty in functioning. Reported psychiatric symptoms included emotional lability (85%), anxiety (64%), depression (51%), spontaneous crying (39%), personality changes (34%), anger/aggression (17%), and psychosis (3%). For most patients (55%), all psychiatric symptoms resolved in parallel with PCS symptoms (mean GAF at recovery = 95); however, for 45% of patients, psychiatric symptoms persisted past the point of recovery from concussion. Among these patients, persisting symptoms included anxiety (66%), depression (42%), anger/aggressiveness (19%), and personality changes (11%). The mean GAF score for these patients at recovery was 80, indicating mild persistent difficulty with functioning for which 85% of this group sought out some form of psychiatric treatment. Conclusion: These results indicate that in as many as one third of persons with concussion, significant psychiatric symptoms may be present during the course of recovery. In most cases, these are likely to resolve in parallel with other concussion symptoms; however for some individuals, further treatment may be required.

Disclosures: M.D. McCrady: None. **S. Rasmussen:** None. **P.I. Rosebush:** None. **M.F. Mazurek:** None.

Nanosymposium

202. Traumatic Brain Injury: Animal and Human Studies

Location: 152A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 202.02

Topic: C.10. Trauma

Support: VA Research Services

Title: The prevalence and impact of sleep disturbances following traumatic brain injury from blast versus non-blast exposure in an oef/oif veteran population

Authors: *J. WATSON[1], K. L. PANIZZON[1,2], N. H. DINH[1], N. SHAH[1], G. V. T. WINDMILLER[1], S. JOO[1], R. A. WALLIS[1,2];
[1]VA Greater Los Angeles Healthcare Syst., Los Angeles, CA; [2]Neurol., David Geffen UCLA Sch. of Med., Los Angeles, CA

Abstract: OBJECTIVE: To evaluate the prevalence of post-traumatic brain injury (TBI) sleep disturbances in subjects with blast versus non-blast TBI in a veteran population from Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF). BACKGROUND: One of the signature injuries from the OEF/OIF conflict is TBI, especially TBI from blast exposure. A number of residual effects may be seen after TBI, which adversely impact recovery. Among these, sleep disturbances can be particularly problematic and further affect neurobehavioral function and overall recovery. METHODS: We conducted a retrospective chart review of patients from the Poly-Trauma Clinic of the VA Greater Los Angeles Healthcare System. We collected data from patients with a confirmed diagnosis of TBI of greater than one year prior. We also collected data regarding sleep disturbances and psycho-social function in subjects with a history of blast versus non-blast exposure. RESULTS: We reviewed a total 527 charts, of which 293 OEF/OIF subjects were identified as having had TBI. The race/ethnic distribution of these subjects was 44 % Caucasian, 12% African-American, 25% Hispanic, 12% Asian, and 7% Other. The mean age of subjects with blast TBI was 33 ± 1 year, while those with non-blast TBI had an age of 32 ± 1 year. Of these OEF/OIF subjects with TBI, we found that 69% ± 3 (n = 201) had suffered blast-exposure, while 31% ± 3 (n = 92) had a non-blast exposure. The overall prevalence of a post-TBI sleep disturbances diagnosed in this population of OEF/OIF subjects and TBI was 82% ± 3 (n = 241). In subjects with post-TBI sleep disturbances, a blast exposure was found in a mean of 83% ± 3 (n = 167) cases, while non-blast exposure was seen in 81% ± 1 (n = 74). CONCLUSION: In this population of OEF/OIF veterans, sleep disturbances occurred frequently in subjects with TBI. The finding of post-TBI sleep disturbance was seen with nearly equal frequency in those subjects with both blast- versus and non-blast exposure. Since sleep disturbances have been shown to have a significant impact upon cognition, mood stability and general wellbeing, the treatment of post-TBI sleep disturbances in OEF/OIF may be a critical issue to be addressed in order to improve overall recovery and long-term health.

Disclosures: **J. Watson:** None. **K.L. Panizzon:** None. **N.H. Dinh:** None. **N. Shah:** None. **G.V.T. Windmiller:** None. **S. Joo:** None. **R.A. Wallis:** None.

Nanosymposium

202. Traumatic Brain Injury: Animal and Human Studies

Location: 152A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 202.03

Topic: C.10. Trauma

Support: VA RR&D Grant 1I01RX000622-01A1

VA RR&D Grant 1IK2RX000709-01A3

Title: Lifetime PTSD confounds diffusion tensor abnormalities associated with military concussion

Authors: ***N. D. DAVENPORT**[1], S. R. SPONHEIM[2], K. O. LIM[2];
[1]Res., Minneapolis Vahcs/University of Minnesota, MINNEAPOLIS, MN; [2]Psychiatry, Minneapolis VAHCS/University of Minnesota, MINNEAPOLIS, MN

Abstract: BACKGROUND: Mild traumatic brain injury (mTBI), also known as concussion, is common among American service members returning from recent overseas combat deployments. However, post-traumatic stress disorder (PTSD) symptoms are also common among these veterans and can make diagnostic differentiation of the two conditions difficult. Previous work has established that disrupted structural connectivity of cerebral white matter is associated with mTBI, but the effect of PTSD on this relationship remains unclear. METHODS: As part of ongoing data collection for a large study of American veterans, clinical (PTSD, mTBI) and diffusion imaging (Mean Diffusivity [MD], Fractional Anisotropy [FA]) measures were collected from 67 American military veterans of deployments to Iraq or Afghanistan who had reported experiences suggesting a possible mTBI during deployment. The effects of blast-related mTBI (bTBI), impact mTBI (iTBI), and their interaction on average integrity (i.e., MD and FA) within 20 standard regions of interest (ROIs), global integrity (i.e., across all white matter voxels), and the number of voxels with abnormally high/low integrity (i.e., >2 SD above/below mean) were tested. RESULTS: When looking only at individuals with no lifetime history of PTSD (n=36), bTBI was associated with lower FA and higher MD in bilateral cingulum and lower global FA, while iTBI was associated with lower FA in left ILF and forceps major, higher MD in 16 of 20 ROIs tested, lower global FA, higher global MD, fewer voxels with high FA or

low MD, and more voxels with high MD. In contrast, when looking only at individuals with a history of PTSD (n=31), no effects of bTBI or iTBI on FA or MD were observed. CONCLUSIONS: In the absence of PTSD, bTBI was preferentially associated with disrupted connectivity in the cingulum, while the effects of iTBI were more widespread, potentially indicating unique mechanisms of damage. The lack of significant effects of mTBI on brain connectivity among individuals with a history of PTSD demonstrates the importance of considering psychological trauma in the evaluation of mTBI.

Disclosures: **N.D. Davenport:** None. **S.R. Sponheim:** None. **K.O. Lim:** None.

Nanosymposium

202. Traumatic Brain Injury: Animal and Human Studies

Location: 152A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 202.04

Topic: C.10. Trauma

Support: VA Research Services

Title: Prevalence of mood lability following traumatic brain injury from blast versus non-blast exposure in a veteran population

Authors: ***N. H. DINH**[1,3], K. L. PANIZZON[2,4], N. SHAH[2], G. V. T. WINDMILLER[2], S. JOO[2], J. WATSON[2], R. A. WALLIS[2,4];
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Abstract: OBJECTIVE: To evaluate the prevalence of post-traumatic brain injury (TBI) emotional lability in Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF) veterans with blast versus non-blast TBI. BACKGROUND: Blast-TBI has been found to be a signature injury in OEF/OIF veterans. Following TBI, a number of sequelae have been shown to occur including neurobehavioral changes. Post-TBI emotional lability can have a profound impact on interpersonal relationships and quality of life. METHOD: We conducted a retrospective chart review of patients with TBI in the Poly-Trauma Clinic of the VA Greater Los Angeles Healthcare System. We collected data regarding emotional lability following blast- versus non-blast exposure in an OEF/OIF veteran population with confirmed TBI. RESULTS: A total of 527 charts were reviewed. Of these, 293 were found to be OEF/OIF veterans with a history of TBI. The racial-ethnic distribution of subjects was 44 % Caucasian, 12% African-

American, 25% Hispanic, 12% Asian and 7% Other. In this group, the age of subjects with blast TBI was found to be a mean 33 ± 1 years and for non-blast TBI, 32 ± 1 years. Of the 293 subjects with TBI, a mean 55 ± 3%(n = 162) were noted to have emotional lability. In these subjects, 57 ± 3% (n = 116) had blast-TBI, while 68 ± 5% (n = 46) had non-blast TBI. CONCLUSION: These data indicate that post-TBI emotional lability is a common occurrence in OEF/OIF veterans with TBI, both with blast-exposure and non-blast exposure. Since mood lability is highly detrimental to quality of life and can interfere with rehabilitative therapies and overall recovery after TBI, effective treatments are needed to help promotional emotional stability.

Disclosures: **N.H. Dinh:** None. **K.L. Panizzon:** None. **N. Shah:** None. **G.V.T. Windmiller:** None. **S. Joo:** None. **J. Watson:** None. **R.A. Wallis:** None.

Nanosymposium

202. Traumatic Brain Injury: Animal and Human Studies

Location: 152A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 202.05

Topic: C.10. Trauma

Support: The William K. Warren Foundation

Title: Peripheral blood inflammatory markers associated with concussion severity and recovery in collegiate athletes

Authors: *R. SINGH[1], K. T. TEAGUE[2,3,4], J. SAVITZ[1,5], D. POLANSKI[6], A. TAYLOR[2], T. B. MEIER[1], P. S. F. BELLGOWAN*[1,5];
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Abstract: Post-concussion blood brain barrier disruption may result in release of trauma-induced inflammatory molecules from the brain into the periphery and provide a target for developing peripheral blood-based inflammatory biomarkers for concussion. Current clinical diagnostic and prognostic methods for concussion recovery rely heavily on subjective clinical judgment and expertise. Premature return-to-play increases the likelihood of repeated concussions, and may be a risk factor for onset of long-term adverse neurological and psychiatric sequelae in athletes.

Development of objective diagnostic tools should enhance diagnostic reliability and decrease the rates of premature return-to-play. A unique profile of inflammatory mediators orchestrate immune responses following trauma, reflecting injury severity and repair processes and thus possesses diagnostic as well as prognostic potential. We hypothesize that levels of inflammatory molecules in the peripheral blood will reflect ongoing brain inflammatory responses post-concussion. Venous blood was collected and Automated Neuropsychological Assessment Metrics (ANAM) was administered to 21 consecutive athletes with a clinical diagnosed concussion at 2 (T1, 1.75±0.64) and 10 (T2, 9.43±1.99) days post injury. Plasma levels of CRP and IL1RA were determined using ELISA and levels of kynurenic acid (KA), 3-Hydroxy kynurenine (3HK) and Quinolinic acid (QUIN) using HPLC with tandem mass spectrometry. Concussion severity inventory (CSI) scores and return-to-play decision length indexed post injury recovery. CSI scores significantly correlated with days withheld from play (ρ=0.65, P=0.004). Plasma levels of IL1RA at T1 significantly correlated with CSI scores (ρ=0.60, P=0.005) and cognitive performance (ρ=-0.47, P=0.042). T1 KA/3HK levels associated positively with CSI scores (ρ=0.42, P=0.074) and an inverse relationship existed between T2 KA/3HK levels and days withheld (ρ=-0.57, P=0.044). These data showed that the degree of initial inflammatory responses including levels of IL1RA and KA/3KH corresponded with both the concussion severity and recovery. Specific blood-based trauma-induced inflammatory mediators are promising as biomarkers to facilitate and aid in clinical validation of the severity of concussions and better inform return-to-play decisions.

Disclosures: **R. Singh:** None. **K.T. Teague:** None. **J. Savitz:** None. **D. Polanski:** None. **A. Taylor:** None. **T.B. Meier:** None. **P.S.F. Bellgowan*:** None.

Nanosymposium

202. Traumatic Brain Injury: Animal and Human Studies

Location: 152A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 202.06

Topic: C.10. Trauma

Support: US Army (USAMRAA): W81XWH-12-C-0205

US IRS (QTDPC): 21.013

Title: The presence of mild traumatic brain injury is reliably detected with a combination of oculomotor and vestibular measures

Authors: *R. C. ASHMORE[1], S. AKHAVAN[2], M. R. QUIGLEY[2], E. D. SNELL[2], R. SCLABASSI[3], L. OAKES[1], A. KIDERMAN[1], K. M. KELLY[2];
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Abstract: As awareness of the long-term consequences of concussion (mild traumatic brain injury, or mTBI) increases, the demand for objective, reliable, non-invasive methods for detecting mTBI becomes more acute. Since previous imaging studies have shown that mTBI is associated with diffuse axonal injury (DIA) in multiple brain pathways, and further studies have indicated influences of mTBI on oculomotor, vestibular, and reaction time functions, we hypothesized that mTBI will present with a diverse, overlapping, but non-homogeneous profile of deficits in these functions, deficits which can be measured non-invasively, reliably, and objectively by video-oculography (VOG). In this study, we used high speed measurements of oculomotor behavior and responses as an objective indicator of the presence of mTBI in patients with mild head injuries. VOG was performed using a head-mounted goggle with two built-in infra-red tracking cameras. We acquired multiple metrics of saccades, smooth pursuit tracking, and nystagmus movements performed during a battery of standard eye movement tests. Also included in the battery were tests for measuring reaction time and vestibular function. These VOG tests were performed on 50 patients and 286 control subjects, and we then constructed a predictive regression model and isolated significantly contributing variables (forward stepwise logistic regression). We found that no one metric was sufficiently predictive to have reliable diagnostic value. Consistent with our hypothesis however, we found 6 metrics that as an aggregate (as features for a logistic regression model) were highly predictive of the presence of a concussion (ROC = 0.97, mean for 20 three-fold cross-validations). These metrics included saccade hypometria, saccadic intrusions in smooth pursuit, smooth pursuit lag, delayed smooth pursuit initiation, impaired optokinetic nystagmus, and alterations in judging true horizontal relative to gravity. Furthermore, the profile of metrics varied across patients, with different relative contributions of the metrics leading to correct concussion determination. These results suggest that concussions indeed produce a broad and non-homogeneous range of neurological deficits and motor/behavioral manifestations, and that video-oculography with multiple tests provides a reliable and accurate method of capturing and quantifying this range for precision diagnostic use.

Disclosures: **R.C. Ashmore:** A. Employment/Salary (full or part-time):; Neuro Kinetics, Inc.. **S. Akhavan:** None. **M.R. Quigley:** None. **E.D. Snell:** None. **R. Sclabassi:** A. Employment/Salary (full or part-time):; Computational Diagnostics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuro Kinetics, Inc. **L. Oakes:** A. Employment/Salary (full or part-time):; Neuro Kinetics, Inc. **A. Kiderman:** A. Employment/Salary (full or part-time):; Neuro Kinetics, Inc.. **K.M. Kelly:** None.

Nanosymposium

202. Traumatic Brain Injury: Animal and Human Studies

Location: 152A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 202.07

Topic: C.10. Trauma

Support: VA Research Services

Title: Post-traumatic headache after blast versus non-blast exposure in an oef/oif veteran population

Authors: *P. J. WELLS[1], K. L. PANIZZON[2,3], N. H. DINH[4], J. WATSON[3], N. SHAH[3], G. V. T. WINDMILLER[3], S. JOO[3], R. A. WALLIS[3,5];
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Abstract: OBJECTIVE: To evaluate the prevalence and severity of post-traumatic brain injury (TBI) headache in subjects with blast versus non-blast TBI in a veteran population deployed with Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF). BACKGROUND: One of the signature injuries for veterans deployed in the OEF and OIF conflicts is that of TBI, particularly blast TBI. Following TBI a wide array of residual effects can be seen, including headache, cognitive dysfunction, irritability and memory loss. METHODS: We conducted a pilot, retrospective chart review of patients with TBI seen at the Poly-Trauma Clinic of the VA Greater Los Angeles Healthcare System. We collected data from patients with a confirmed diagnosis of TBI from whom TBI had occurred greater than one year prior. We also examined headache severity in these subjects. Headache severity was categorized as mild, moderate and severe according to a 1 to 4 pain scale. RESULTS: A total of 527 charts were reviewed. Within these, 293 were identified as OEF/OIF subjects with a confirmed diagnosis of TBI. The racial/ethnicity background of OEF/OIF subjects with TBI was 44 % Caucasian, 12% African-American, 25% Hispanic, 12% Asian, and 7% Other. We found that 69% ± 3 (n = 201) of subjects had suffered blast-TBI and 31% ± 3 (n = 92) non-blast TBI. The mean age of subjects with blast TBI was 33 ± 1 years and of those with non-blast TBI, 32 ± 1 years. Post-TBI headache was diagnosed in a mean of 78% ± 3 (n = 156) subjects with blast exposure and 75% ± 4 in non-blast TBI. The prevalence of severe headaches was 55% ± 4 in subjects with blast TBI and 42% ± 6 in those with non-blast TBI. CONCLUSION: In this population of veterans with TBI who had served in OEF/OIF, post-TBI headache was reported frequently following blast and

non-blast TBI. However, post-TBI headache showed significantly greater severity in OEF/OIF veterans with blast-TBI than those with non-blast TBI. These initial data suggest that blast TBI in OEF/OIF veterans may be associated with more frequent and severe post-TBI headache. They also suggest that post-TBI headache may have greater headache severity for patients with blast-exposure than that seen with non-blast exposure.

Disclosures: **P.J. Wells:** None. **K.L. Panizzon:** None. **N.H. Dinh:** None. **J. Watson:** None. **N. Shah:** None. **G.V.T. Windmiller:** None. **S. Joo:** None. **R.A. Wallis:** None.

Nanosymposium

202. Traumatic Brain Injury: Animal and Human Studies

Location: 152A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 202.08

Topic: C.10. Trauma

Support: VA RRD Merit

CURE Taking Flight Award

Title: Disruptions in synaptic plasticity in the hippocampus across a spectrum of mild traumatic brain injury levels in swine

Authors: ***J. A. WOLF**[1,2], A. V. ULYANOVA[1], M. R. GROVOLA[1,2], J. P. HARRIS[1,2], K. D. BROWNE[1], V. E. JOHNSON[1], D. H. SMITH[1], J. E. DUDA[2], D. K. CULLEN[1,2];
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Abstract: Mild traumatic brain injury (TBI) as currently defined covers a relatively large spectrum of acute neurological outcomes ranging from no loss of consciousness (LOC) to LOC lasting just under 30 minutes. Moreover, long-term outcomes also vary as 15-20% of individuals sustaining a mild TBI exhibit persistent cognitive deficits. The objective of the current study was to assess functional and structural alterations in brain circuitry using a model of closed-head non-impact inertial TBI in swine following a range of "mild" injuries. Yucatan mini-pigs (~30kg) were subjected to sham conditions or rapid head rotation in the coronal plane at peak angular velocities of 180, 220, or 260 rad/sec. These injury levels all induce clinically defined mild TBI based on LOC and neurological recovery, absent subdural hemorrhage but with diffuse axonal injury in sub-cortical white matter tracts. At 7 days post-injury, we examined neurophysiological

changes in the hippocampus using in vivo electrophysiological recordings with high-density recording arrays and simultaneous afferent stimulation, with a focus on hippocampal synaptic responses. In particular, multi-electrode electrophysiological recordings (linear, 32-channels) were performed in the dorsal hippocampus, and concentric bipolar stimulation was performed in Schaffer collaterals and the entorhinal cortex, using either paired-pulse or theta-burst paradigms. Input-output curves were generated and paired-pulse paradigms were utilized to examine changes in neurotransmitter release probabilities. Changes in synaptic strength after post-tetanic stimulation were visualized pre and post theta-burst stimulation to examine changes in potentiation post injury. CSD analysis was utilized to examine changes in synaptic inputs to hippocampal layers. These experimental paradigms revealed a graded loss of paired pulse facilitation in area CA1 by 7 days post injury, potentially due to changes in neurotransmitter release probability, which varied based on mild TBI level. Changes in the post-tetanic potentiation were also noted, with a loss of potentiation in injured compared to sham animals at all injury levels. These data indicate that mild TBI in swine leads to dysfunction in various aspects of hippocampal synaptic function post-injury, potentially underlying cognitive dysfunction. Further histopathological examination may indicate substrates for these synaptic changes post-injury. These data suggest graded neurophysiological alterations in synaptic function that vary based on degree of diffuse brain injury in swine, and that further stratification of the mild TBI classification may be necessary.

Disclosures: **J.A. Wolf:** None. **A.V. Ulyanova:** None. **M.R. Grovola:** None. **J.P. Harris:** None. **K.D. Browne:** None. **V.E. Johnson:** None. **D.H. Smith:** None. **J.E. Duda:** None. **D.K. Cullen:** None.

Nanosymposium

202. Traumatic Brain Injury: Animal and Human Studies

Location: 152A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 202.09

Topic: C.10. Trauma

Support: CONICYT

Biomedical Neuroscience Institute

ICM P10-001-F, P09-015-F

Title: Transitory, fast changes in theta/alpha band EEG signals, in vegetative state patients during responses to complex stimuli

Authors: *G. RIVERA[1], J. I. EGAÑA[2], V. DIAZ[3], P. MALDONADO[4];
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Abstract: The vegetative state is a conscious disorder characterized by lack of a sustained, reproducible or voluntary behavioral responses to sensory stimulation. Previous reports have showed that subjects in this condition form a heterogeneous group, presenting different evoked and oscillatory response to different complexity stimuli. This remnant activity could be associated with different processing abilities and can be related to the indemnity of some brain regions, or to remaining connectivity between cortico-cortical and cortico-thalamic structures. One way in which these cortico-thalamic connections favor the functional cortical integration is in the generation and temporal modulation of alpha band activity, which is related to a wide range of perceptual and cognitive functions. We propose that transitory changes in alpha (power spectrum) in response to auditory stimuli depend on the different complexity stimuli and reflect remnant connectivity in a vegetative state. We recorded data from six subjects in vegetative states and ten control subjects. We measured the evoked and oscillatory activity (24-Channel EEG) to auditory stimuli using a classical oddball paradigm where the deviant stimulus was the patient's own name pronounced by a family member (emotional valence). We observed variable features in the evoked activity for the vegetative state patients. We found significant fast changes in alpha/theta band power spectrum that can be seen in response to deviant stimulus in some to the vegetative patients. Similar to previously reported in normal subjects, most of the vegetative state patients showed a decrease of alpha power after the deviant stimuli, mostly seen in Cz, around 400-900ms. These results contribute to the model that proposes a lack of cortical integration due to loss of functional connectivity between different cortical areas. Despite the absence of evoked response, is possible observe different dynamics in the alpha band in vegetative state for emotional (deviant) stimuli, which can reflect different degrees of remnant connectivity. Supported by ICM P10-001-F, P09-015-F and Conicyt for GR

Disclosures: **G. Rivera:** None. **J.I. Egaña:** None. **V. Diaz:** None. **P. Maldonado:** None.

Nanosymposium

202. Traumatic Brain Injury: Animal and Human Studies

Location: 152A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 202.10

Topic: C.10. Trauma

Support: NIH 2T35OD012199-11A1

ISU CVM Seed Grant

Title: Analysis of neuronal trauma and micro-tears in a rodent traumatic brain injury model

Authors: G. MLYNARCZYK, *D. C. PETERSON;
Biomed Sci., Iowa State Univ., Ames, IA

Abstract: Exposure of military personnel to blast-pressure waves have increased within the last 10 years. These exposures induce traumatic brain injuries which can manifest in learning or memory deficits, post-traumatic stress disorder, or attention deficit disorder. To examine the histological changes that occur due to a blast exposure we assessed both Nissl- and GABAergic stained brain sections from a traumatic brain injury rodent model. Brain sections were examined for occurrences of micro-tears and areas of gross cellular damage following exposure to a 20 psi supersonic pressure wave directed from rostral to caudal. Neuronal degeneration was observed in discrete regions of the brain. A majority of degeneration was observed in the midbrain (medial parabrachial nuclei, ventrolateral tegmental region) and cortex (dentate gyrus, amygdala, and cortico-amygdalar transition zones. Lesser degrees of degeneration were observed in the Islands of Calleja and ventral pallidum. Regions with major micro-tear damage were primarily within cortex, the forebrain, and amygdala. Within cortex, damage was focused in auditory, somatosensory, piriform, and retrosplenial cortex. Smaller regions of more dispersed damage were also observed in motor and visual cortices. The location of micro-tears was more prominent in rostral sections of the brain and decreased in frequency as we moved caudally through the brain sections. Many micro-tears were dispersed throughout the affected brain regions; however clumps of tears were also noted. Both concentrated and dispersed regions of micro-tears were observed throughout all layers of cortex. Micro-tears ranged in size from small (3 μm) to large (25 μm). Sizes of micro-tears did not appear to depend on brain location, however a larger proportion of tears were observed near GABAergic neurons. The results correlate with behavioral phenotypes (e.g., tinnitus formation and depression) that are exhibited by blast-exposed animals in our model. Because the rodent behavioral data mimics the symptomology of blast-exposed humans, we hypothesize that micro-tears within cortical and amygdalar brain regions alter the function of various circuits in these regions. These alterations can potentially explain the myriad symptomology observed after blast-exposure in both rodent and human populations.

Disclosures: G. Mlynarczyk: None. **D.C. Peterson:** None.

Nanosymposium

202. Traumatic Brain Injury: Animal and Human Studies

Location: 152A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 202.11

Topic: C.10. Trauma

Support: CDMRP funding (Crawford W81XWH-10-1-0759)

VA Merit funding (Crawford)

the Roskamp Foundation

Title: Two year plasma lipidomic profile in a mouse model of single and repetitive mild Traumatic Brain Injury

Authors: *F. C. CRAWFORD[1,2,3], B. MOUZON[1,2,3], T. EMMERICH[1,3], L. ABDULLAH[1,4], J. EVANS[1,2], J. M. REED[1,2], G. C. CRYNEN[1,2], M. J. MULLAN[1,2,3];
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Abstract: Traumatic brain injury (TBI), in particular mild TBI (mTBI) is a major problem for both military and civilian populations. An objective panel of biomarkers for TBI and related conditions would enable appropriate medical management, may indicate ongoing pathogenic processes, provide guidance in therapeutic development, and could be used to monitor outcome and response to treatment. We have developed a mouse model of single and repetitive mTBI that shows progressive neuroflammation and neurobehavioral changes, characterized through to 2 years post injury. Phospholipids (PLs) such as phosphatidylcholine (PC) and sphingomyelin (SM), play a prominent role in neuronal processes including neurotransmitter release, neurite outgrowth and synaptogenesis, and brain lipid metabolism is disrupted in our preclinical TBI models. We have used our lipidomic platform (in-source collision induced dissociation (sCID) with full scan liquid chromatography/MS (LC/MS)) to generate a temporal profile of plasma lipidomic changes in our mouse model. Plasma profiling demonstrates significant TBI-dependent changes in lipid profiles that persist years after the injury, including significant increases in PC and SM. To determine the clinical relevance of these findings we will correlate plasma lipidomic changes with brain lipidomic changes in this model, but we are also validating our findings by investigating plasma lipid profiles in human TBI populations. Funding CDMRP funding (Crawford W81XWH-10-1-0759), and VA Merit funding (Crawford); and the Roskamp Foundation.

Disclosures: **F.C. Crawford:** None. **B. Mouzon:** None. **T. Emmerich:** None. **L. Abdullah:** None. **J. Evans:** None. **J.M. Reed:** None. **G.C. Crynen:** None. **M.J. Mullan:** None.

Nanosymposium

202. Traumatic Brain Injury: Animal and Human Studies

Location: 152A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 202.12

Topic: C.10. Trauma

Support: New Jersey Commission on Brain Injury Research, multi- investigator grant - CBIR11PJT003

Title: Impact of brain injury on high frequency cerebellar oscillations in rat

Authors: ***G. ORDEK**[1], A. PRODDUTUR[2], V. SANTHAKUMAR[2], B. PFISTER[1], M. SAHIN[1];
[1]Biomed. Engin., New Jersey Inst. of Technol., Newark, NJ; [2]Dept. of Neurol. and Neurosciences, Rutgers Biomed. and Hlth. Sci., Newark, NJ

Abstract: Traumatic brain injuries, particularly mild cases (concussions), are clinically important, as evidenced by the morbidity rate in sports and traffic accidents, yet lack an accurate technique to assess neurological impairment. Conventional diagnostic EEG, quantitative EEG (QEEG) or neuroimaging methods have been used successfully to identify brain injuries in victims of severe impacts. Typical brain injuries present two phases; a primary phase due to mechanical distress and a secondary phase that lasts from days to months. Latter is the main focuses of brain researchers, since the primary injuries can be prevented only by safety precautions. To this purpose, animal studies constitute a viable framework to investigate the development of delayed injury-mechanism in a controlled manner. Here we use a subdural electrophysiology (ECoG) technique to detect-injury related alterations in time (Immediate – 7 Days) in a highly organized brain structure; cerebellum. Rats were chronically implanted with micro ECoG electrodes (31-channels) on the paramedian lobule. Fluid percussion injury (FPI) was delivered ipsilateral site to the implant. Animals were subjected to 10-day recording sessions including three pre-injury and seven post-injury days under ketamine-xylazine anesthesia and during wakefulness. Immunohistology on cerebellar slices was used to confirm neuronal degeneration after injury. Post-FPI recordings (Immediate and Day 7) were compared with baseline recordings during spontaneous as well as in peripherally evoked potentials. Immediate recordings indicated a rapid elevation in the power of low-frequency oscillations (< 30Hz). At

day-1, there was substantial deterioration in the amplitude and frequency of the cerebellar signals. The averaged cross-correlations decreased to r = ~0.1-0.2 ($r_{baseline}$ =0.4-0.5) and r = ~0.3-0.4 ($r_{baseline}$ = 0.6-0.9) for spontaneous and evoked potentials in the high gamma frequency band (80-200Hz), respectively. Mean-Correlation across all contacts continued to decrease in the following days of FPI and was $r \leq 0.1$ at the end of the survival period (Day 7). Interestingly, the trend towards a decline in the average inter-contact coherence was 30-50% larger in electrode groups aligned in transverse rather than sagittal direction, suggesting spatial orientation of the injury effect. Additionally, evoked potential amplitudes were also reduced substantially to 20-30±10% of baseline amplitudes obtained in pre-injury recordings. Thus, electrophysiological assessment of the cerebellum can reveal quantitative information about traumatic brain injuries in both spatial and temporal domains.

Disclosures: G. Ordek: None. **A. Proddutur:** None. **V. Santhakumar:** None. **B. Pfister:** None. **M. Sahin:** None.

Nanosymposium

202. Traumatic Brain Injury: Animal and Human Studies

Location: 152A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 202.13

Topic: C.10. Trauma

Support: This project was partially funded through a grant from the Depart- ment of Veterans Affairs, Veterans Health Administration, Reha- bilitation Research and Development Ser

Title: Behavioral outcomes differ between repeated shockwave and head rotational acceleration exposures

Authors: *B. D. STEMPER, A. SHAH, M. D. BUDDE, M. MCCREA, S. N. KURPAD, A. GLAVASKI-JOKSOMOVIC, F. A. PINTAR;
Dept. of Neurosurgery, Med. Col. of Wisconsin, Milwaukee, WI

Abstract: TBI can result from rotational or blast mechanisms. Although treatment and rehabilitation strategies are not altered based on mechanism, evidence of different outcomes between blast and rotational TBI imply that these injuries are not identical and may require custom interventions. This study quantified differences in behavioral outcomes following single or repeated exposure to mild TBI through head rotational acceleration (SR, RR) or blast (SB, RB). Anesthetized Sprague-Dawley rats were exposed to either single or repeated (n=2)

exposures to either head rotational acceleration or shockwave. Rats receiving two injuries were exposed on day 0 with a second exposure two days later. Rats receiving a single injury were exposed on day 2. Morris Water Maze (MWM) and Elevated Plus Maze (EPM) assessments were conducted on days 5-9. MWM identified cognitive deficits with latency to find the platform significantly greater ($p<0.05$) for SB and RB compared to shams during the 3rd set (Day 8). The number of unsuccessful trials demonstrated a progressive increase from shams to SR to RR groups. EPM showed blast and rotational exposures resulted in greater post-injury activity compared to shams, with single and repeated exposures resulting in increased total arm changes. However, SR and RR led to a significant and progressive increase, with post-hocs demonstrating a significant increase for the RR group compared to shams ($p<0.05$). SB and RB showed similar significant increases in open arm time compared to controls ($p<0.05$). However, rotational acceleration resulted in different trends, with a progressive increase in open arm time compared to shams. The magnitude of increase was greater following SB than SR, which may indicate more severe emotional changes following blast TBI. This preliminary study demonstrated clear and consistent differences in cognitive and emotional outcomes following single and repeated TBI resulting from blast or rotational acceleration, which highlights the role of mechanism on outcomes following TBI. The results demonstrated more severe outcomes following initial exposure to blast, but a cumulative effect of head rotational accelerations that may increase the severity of outcomes with accumulating exposures.

Disclosures: B.D. Stemper: None. **A. Shah:** None. **M.D. Budde:** None. **M. McCrea:** None. **S.N. Kurpad:** None. **F.A. Pintar:** None. **A. Glavaski-Joksomovic:** None.

Nanosymposium

202. Traumatic Brain Injury: Animal and Human Studies

Location: 152A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 202.14

Topic: C.10. Trauma

Support: DOD (W81XWH-12-1-0629)

NINDS (NS082798)

NIA (AG023012)

NINDS (NS074804)

Title: Traumatic brain injury induces a distinct macrophage response at acute and chronic time points in a mouse model of Alzheimer's disease

Authors: *O. KOKIKO-COCHRAN[1], M. SABER[2], R. TEKNIPP[2], R. RANSOHOFF[2], B. LAMB[2];
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Abstract: Increasing evidence implicates traumatic brain injury (TBI) as a major risk factor for a number of neurodegenerative diseases, including Alzheimer's disease (AD). Neuroinflammation is an early hallmark feature of both TBI and AD, and we hypothesize that the brain injury induced inflammatory response is critical in promoting AD-like phenotypes. To test this hypothesis, lateral fluid percussion TBI or sham injury was administered to a genomic based model of beta-amyloid deposition (R1.40) in which the *APP* gene is expressed under the control of endogenous human regulatory elements as well as non-transgenic controls. Initial studies demonstrated that TBI resulted in a large injury cavity accompanied by marked microglial activation in both genotypes at acute postinjury time points; however, there was a surprising reduction in the presence of activated macrophages near the site of injury in brain injured R1.40 mice compared to brain injured control mice. Subsequent studies have utilized $Cx3cr1^{GFP/GFP}/Ccr2^{RFP/RFP}$ mice to generate heterozygous $Cx3cr1^{GFP/+}/Ccr2^{RFP/+}$ mice with or without R1.40 to characterize the spatial distribution of microglia, tagged with GFP, and monocytes, tagged with RFP, after TBI. Activated GFP+ microglia and infiltrating RFP+ monocytes were identified in close spatial proximity to the injury cavity in both R1.40 and control brain injured mice at 3 days postinjury (DPI). Surprisingly, RFP+ monocytes were detected in cortical as well as subcortical brain regions at a chronic postinjury time point of 120 DPI, where a sustained macrophage response was identified in R1.40 brain injured mice in our initial studies. Microglial activation notably decreased by 120 DPI in both injured groups. In summary, these experiments suggest that the presence of accumulating Aβ in the R1.40 mice compromises the macrophage response to a secondary immune challenge such as TBI at both acute and chronic time points with potentially distinct effects on microglia and monocytes. Ongoing experiments are examining the phenotypes and gene expression profiles of both resident microglia and infiltrating monocytes at acute and chronic postinjury time points.

Disclosures: O. Kokiko-Cochran: None. **M. Saber:** None. **R. Teknipp:** None. **R. Ransohoff:** None. **B. Lamb:** None.

Nanosymposium

203. Obstructive Sleep Apnea: Intermittent Oxygenation

Location: 152B

Time: Sunday, November 16, 2014, 1:00 PM - 2:30 PM

Presentation Number: 203.01

Topic: E.04. Autonomic Regulation

Support: National Institutes of Health R01 HL-113251.

Title: Association between brain injury and disease severity in patients with obstructive sleep apnea

Authors: *S. K. YADAV[1], J. PALOMARES[1], M. A. WOO[2], D. W. KANG[3], R. M. HARPER[4,5], R. KUMAR[1,5,6];
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Abstract: Obstructive sleep apnea (OSA) is characterized by repetitive pauses in breathing during sleep, while diaphragmatic efforts continue. Brain structural injury occurs in OSA subjects in multiple sites, which are involved regulating various autonomic, cognitive, motor, and respiratory functions. However, brain tissue changes with OSA severity are unknown. Our aim was to assess relationships between diffusion tensor imaging (DTI)-based regional brain mean diffusivity values and apnea-hypopnea index (AHI) scores (disease severity of OSA) in OSA subjects. We performed DTI (two separate series; 64 diffusion directions and 7 b0 images), using a 3.0 Tesla MRI scanner, in 17 newly-diagnosed, treatment-naive OSA subjects (age, 49.8±9.5 years; 4, females; AHI, 40.8±23.4 events/hour; body mass index, 29.4±8.9 kg/m2). Using DTI data, MD maps were derived from each series, realigned, averaged, normalized to a common space, and smoothed, and correlations between regional MD values and AHI scores were examined using linear regression analysis (covariates; age and gender; uncorrected threshold; $p = 0.005$). Positive correlations between regional MD values and AHI scores emerged in the inferior frontal cortices, ventral and superior temporal gray matter, cingulate and insular cortices, amygdala, parahippocampal gyrus, and caudal pons, and negative correlations emerged in the putamen, occipital cortices, cerebellar peduncle, cerebellar cortices and deep nuclei, globus pallidus, and hypothalamus. The findings of negative and positive correlations between regional MD values and AHI scores suggest both acute and chronic tissue changes, respectively. The longer-term changes suggest that autonomic regulatory areas, e.g., insula, inferior frontal cortices, cingulate, are affected earlier, with basal ganglia and cerebellar structures affected later. Supported by National Institutes of Health R01 HL-113251.

Disclosures: **S.K. Yadav:** None. **J. Palomares:** None. **M.A. Woo:** None. **D.W. Kang:** None. **R.M. Harper:** None. **R. Kumar:** None.

Nanosymposium

203. Obstructive Sleep Apnea: Intermittent Oxygenation

Location: 152B

Time: Sunday, November 16, 2014, 1:00 PM - 2:30 PM

Presentation Number: 203.02

Topic: E.04. Autonomic Regulation

Support: Texas Garvey Foundation

NIH P01 HL88052

NIH T31 AG0204

Title: Intermittent hypoxia and its role in oxidative stress and inflammation

Authors: *B. SNYDER[1], B. SHELL[2], J. CUNNINGHAM[2], R. L. CUNNINGHAM[2]; [1]Univ. of North Texas Hlth. Sci. Ctr., FT WORTH, TX; [2]Univ. of North Texas Hlth. Sci. Ctr., Ft. Worth, TX

Abstract: Inflammation has been linked with sleep apnea. Sleep apnea is a common comorbidity associated with neurodegenerative disorders, such as Parkinson's disease and Alzheimer's disease. Furthermore, neurodegenerative diseases have also been linked with inflammation. A possible mechanism underlying increased inflammation in these disorders is oxidative stress, a hallmark of neurodegeneration. To examine the role of oxidative stress on inflammation, we used chronic intermittent hypoxia (CIH), an established model for the hypoxemia associated with sleep apnea. CIH consists of recurring events of low oxygen followed by reoxygenation. We hypothesize that CIH causes oxidative stress, which induces inflammation. To test this hypothesis, plasma from adult male rats subjected to 7 days of CIH (3 minute periods of hypoxia (10% oxygen) and 3 minute periods of normoxia (21% oxygen) for 8 hours per day) or normoxia (room air) were tested for AOPP, an indicator of oxidative stress, and circulating inflammatory markers (IL-1β, IL-10, IL-4, IL-6). Additionally, a group of rats was administered a neurotropic AAV with shRNA for AT1a receptors in their forebrains and instrumented with telemetry for blood pressure recording prior to CIH treatment to determine the effects of angiotensin on CIH hypertension and oxidative stress. Our results showed that CIH significantly increased circulating oxidative stress and inflammation. Interestingly, the neuronal IL-1β, IL-4, and IL-10 inflammatory markers were associated with oxidative stress, unlike the macrophage IL-6 inflammatory marker. Knockdown of angiotensin 1 receptors in the forebrain blocked the diurnal hypertension and CIH induced oxidative stress, indicating the involvement of CIH hypertension and central angiotensin receptors in CIH induced oxidative stress. These results indicate that both neurons and macrophages contribute to CIH induced oxidative stress and inflammation and that

CIH oxidative stress and inflammation is dependent on central angiotensin receptors and CIH hypertension.

Disclosures: **B. Snyder:** None. **B. Shell:** None. **J. Cunningham:** None. **R.L. Cunningham:** None.

Nanosymposium

203. Obstructive Sleep Apnea: Intermittent Oxygenation

Location: 152B

Time: Sunday, November 16, 2014, 1:00 PM - 2:30 PM

Presentation Number: 203.03

Topic: E.04. Autonomic Regulation

Support: National Institutes of Health R01 HL-113251.

Title: Extent of oxygen desaturation affects brain tissue pathology in obstructive sleep apnea

Authors: J. PALOMARES[1], S. K. YADAV[1], D. W. KANG[2], M. A. WOO[3], *R. K. HARPER[4], R. M. HARPER[4,5], R. KUMAR[1,5,6];
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Abstract: Obstructive sleep apnea (OSA) is characterized by loss of upper airway muscle tone, with continued diaphragmatic efforts, resulting in repeated airway collapse and obstruction, and intermittent hypoxic periods. OSA patients show brain structural injury, based on various MRI procedures, in multiple regions; however, pathological mechanisms contributing to tissue changes are unclear. The repeated intermittent desaturations can contribute to brain tissue changes, and such contributions can be assessed by examining relationship between diffusion tensor imaging (DTI) based mean diffusivity (MD) values and O2 saturation nadirs. We performed DTI (two series; 64 diffusion directions and 7 b0 images), using a 3.0 Tesla MRI scanner, in 17 newly-diagnosed, treatment-naive OSA (age, 49.8±9.5 years; 4, males; AHI, 40.8±23.4 events/hour; body mass index, 29.4±8.9 kg/m2; O2 saturation nadir change, 14.8±7.5%). Using DTI data, MD maps were derived from each series; both maps were realigned, normalized to a common space, and smoothed MD maps were used to examine correlations between regional MD values and O2 saturation nadir change with linear regression analysis (covariates; age and gender; uncorrected threshold; $p<0.005$). Positive correlations between MD and O2 saturation change values emerged in the mid corona radiata, cingulate cortices, parietal cortices, inferior and middle frontal cortices, extending to white matter,

temporal gray and white matter, and left occipital cortices, and cerebellar vermis, and negative correlations appeared in the cingulate, thalamus, anterior limb of internal capsule extending to putamen, splenium, and right occipital cortices. Both negative and positive correlations between regional brain MD and O2 saturation nadir change values, indicating acute and chronic tissue changes in those areas, appear in OSA subjects. The extent of pathology in brain tissue with OSA apparently partially depends on the extent of O2 desaturation, and the effects represent both chronic and continued development. Supported by National Institutes of Health R01 HL-113251.

Disclosures: J. Palomares: None. **S.K. Yadav:** None. **D.W. Kang:** None. **M.A. Woo:** None. **R.K. Harper:** None. **R.M. Harper:** None. **R. Kumar:** None.

Nanosymposium

203. Obstructive Sleep Apnea: Intermittent Oxygenation

Location: 152B

Time: Sunday, November 16, 2014, 1:00 PM - 2:30 PM

Presentation Number: 203.04

Topic: E.04. Autonomic Regulation

Support: Alberta Innovates Health Solutions (AIHS) 2013 Summer Studentship

Women and Children's Health Research Institute (WCHRI) 2013 Summer Studentship

CIHR Grant RES0006842

Title: The role of adenosine in the hypoxic ventilatory response

Authors: *N. Y. CHU[1,2], Y. ZHANG[1,2], T. S. ALVARES[1,2], J. D. YOUNG[1,2], C. E. CASS[1,3], G. D. FUNK[1,2];
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Abstract: The ventilatory response to hypoxia is characterized by an initial phase 1 increase followed by a secondary depression, during which minute ventilation (V_E) remains above control in adults, but falls below baseline in newborn and premature mammals. This depression can be life-threatening. Hence there is great interest in underlying mechanisms. Extracellular adenosine (ADO) levels increase during hypoxia via transport from intracellular stores or degradation of extracellular ATP. ADO has long-been hypothesized as a major contributor to the respiratory depression, but data are equivocal. ATP is released during hypoxia where its excitatory actions attenuate the secondary depression. ATP, however, is rapidly degraded into ADO by enzymes called ectonucleotidases. Thus, while ATP is excitatory, its by-product ADO is inhibitory. An

additional source of extracellular ADO is via transport from intracellular stores by equilibrative nucleoside transporters, ENT1 and ENT2 that move ADO across cell membranes along its concentration gradient. Our objectives were to test the hypotheses that ADO contributes to hypoxic respiratory depression and that the ADO underlying this inhibition derives from degradation of extracellular ATP. We measured, via whole-body plethysmography, the hypoxic ventilatory response of wild-type, ENT1 and ENT2 knockout (KO) mice, and ENT1/2 double KO mice. Breathing was recorded for 10 min in room air, 10 min in hypoxia (8% O_2, balance N_2), and 10 min of room air. The magnitude of hypoxic depression was calculated by comparing ventilatory parameters at the peak of the initial increase with the steady-state level attained during the secondary depression. ENT2 KOs were no different that wild-type controls. The secondary depression of V_E in ENT1 KOs (44.0±4.8%, n=6) was significantly greater than wild types (30.3±2.7%, n=8) and ENT2 KOs (26.6±6.1%, n=7). Most dramatically, the depression was 100% in ENT1/2 double KO mice; i.e. V_E returned to baseline. This in part reflected that double KOs showed a much smaller phase 1 increase in V_E; the increase peaked at 161±29%, 225±45% and 187±34% for WT, ENT1 and ENT2 KO mice, respectively but was only 32±8% for the double KOs (n=7); tidal volume fell with hypoxia in the double KOs but increased in all other groups. Data suggest that extracellular ADO is a major contributor to the secondary hypoxic respiratory depression and that its removal by ENT1 normally attenuates the depression. Finally, enhanced respiratory depression in ENT1 and double KOs suggests that the ADO underlying the inhibition derives from the degradation of extracellular ATP, not the transport of ATP from intracellular stores.

Disclosures: **N.Y. Chu:** None. **Y. Zhang:** None. **T.S. Alvares:** None. **J.D. Young:** None. **C.E. Cass:** None. **G.D. Funk:** None.

Nanosymposium

203. Obstructive Sleep Apnea: Intermittent Oxygenation

Location: 152B

Time: Sunday, November 16, 2014, 1:00 PM - 2:30 PM

Presentation Number: 203.05

Topic: C.08. Ischemia

Support: NIH Grant R01HL104173

Baier Cardiac Research Fund

Title: Subventricular zone of the developing gyrencephalic brain under normal physiological conditions and after hypoxia

Authors: *N. ISHIBASHI[1,2], P. D. MORTON[2], L. KOROTCOVA[1], K. AGEMATSU[1], R. A. JONAS[1], V. GALLO[2];
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Abstract: The majority of children with congenital heart disease (CHD) suffer from neurodevelopmental delay as a consequence of their CHD. Reduced oxygen delivery due to cardiac anomalies *in utero* results in sub-normal cortical development. Chronic exposure to hypoxia alters neuronal/glial cell development. Thus, optimal treatment requires new therapies aimed at regenerating injured cells. Neural stem/progenitor cells (NSPCs) retain their mitotic and differentiation potential, as the brain is able to replenish damaged neurons and glial cells. The largest source of these cells is the subventricular zone (SVZ). However, the structural and cellular properties of the well-studied rodent SVZ are very different from its human counterpart. Because of obvious technical/ethical hurdles, the contribution of the SVZ to development of the gyrencephalic human brain and the response of these cells to pathological environments remain poorly understood. This prevents advancing regenerative treatments for neonatal hypoxic brain damage. Thus, development of human-like SVZ NSPCs needs to be defined in the gyrencephalic brain under normal and pathological conditions. The developing porcine brain displays features that are similar to the human brain, including gyrencephalic, rather than smooth, cortex and a similar progression in white matter maturation. Here we demonstrate that in early postnatal period: i) the anatomical and cellular characteristics of the porcine SVZ closely resemble their human counterpart; ii) the dorsolateral-SVZ contains the highest number of NSPCs and is a predominant proliferative region; and iii) during normal development, porcine SVZ NSPCs migrate into the cortex. We also established a hypoxic injury paradigm in piglets. In this model, hypoxia causes a reduction in the NSPC pool of the human-like porcine SVZ. Cell proliferation and neurogenesis in the SVZ also decrease after hypoxia. In cortex, hypoxia results in a significant reduction in neuroblasts. However, increased apoptosis and apoptotic cortical neuroblasts are not observed, suggesting that a decrease in cell generation from the SVZ directly results in a reduction of the cortical neuroblast number. Finally, macro-structural alterations of the developing porcine brain, due to perinatal hypoxia, are very similar to those observed in CHD newborns. Altogether, our findings suggest that: i) SVZ NSPCs contribute to postnatal gyrencephalic cortical development; and ii) hypoxia-induced reduction in neurogenesis from NSPCs alters cortical development. Therefore, alterations of NSPCs of the SVZ may represent a major cellular mechanism underlying sub-normal cortical development in CHD.

Disclosures: **N. Ishibashi:** None. **P.D. Morton:** None. **L. Korotcova:** None. **K. Agematsu:** None. **R.A. Jonas:** None. **V. Gallo:** None.

Nanosymposium

203. Obstructive Sleep Apnea: Intermittent Oxygenation

Location: 152B

Time: Sunday, November 16, 2014, 1:00 PM - 2:30 PM

Presentation Number: 203.06

Topic: E.04. Autonomic Regulation

Support: CIHR Grant RES0018140

WCHRI

AIHS

CFI

ALA

NIH Grant R01HL089742

Title: XII inspiratory premotoneurons derive from a subpopulation of Dbx1 neurons in the intermediate reticular formation

Authors: *A. L. REVILL[1], A. KOTTICK[2], V. T. AKINS[2], N. C. VANN[2], P. A. GRAY[3], C. A. DEL NEGRO[2], G. D. FUNK[1];
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Abstract: Rhythmic activation of the genioglossus muscle (GG), a tongue protruder, plays a key role in maintaining airway patency during inspiration. Reduced tonic and inspiratory activity in XII motoneurons (MNs) that innervate the GG is strongly implicated in obstructive sleep apnea. During sleep, reduced activity is hypothesized to result from loss of excitatory monoaminergic modulation, inhibitory muscarinic modulation, active inhibition and loss of glutamatergic inspiratory drive, which derives from the preBötzinger Complex (preBötC; generates the drive) via inspiratory XII preMNs (transmit drive from preBötC to XII MNs). PreBötC inspiratory drive appears minimally affected by sleep state as phrenic MN output, also derived from the preBötC, is relatively insensitive to sleep state. In contrast, reduced excitability and output of glutamatergic inspiratory preMNs may contribute significantly to the reduced activity of XII MNs during sleep. Unfortunately, little is known about inspiratory preMNs, which are sparsely distributed in the medullary intermediate reticular formation (IRt) and are difficult to distinguish from other neurons. Brainstem interneurons whose progenitors express the transcription factor Dbx1 are largely glutamatergic, a subpopulation of which is present in the IRt. We hypothesized

that ipsilaterally-projecting XII inspiratory preMNs derive from Dbx1 progenitors. Rhythmic, transverse medullary slices (550 μm) were generated from *Dbx1*^{ERCreT2}*; R26*^{tdTomato} neonatal mice (postnatal day 0-5). Inspiratory-related activity was recorded from the XII nerve. Dbx1 neurons in the IRt were examined for inspiratory activity using calcium imaging, on-cell and whole-cell recording methods. Rhythmic calcium oscillations (n=2) and synchronous action potential bursts (on-cell recording, n=6) recorded from IRt Dbx1 neurons were in phase with XII motor output. Whole-cell recording revealed 23 inspiratory-modulated IRt Dbx1 neurons. Antidromic activation from the ipsilateral XII nucleus was confirmed in 8 of 18 neurons. Anatomical reconstructions of biocytin-filled neurons revealed 3 with commissural axons, and 2 with ipsilateral projections towards the XII nucleus. Neurons were characterized electrophysiologically as follows: input resistance (n=10), 403±56 MΩ; rheobase (n=10), 152±24 pA; and membrane time constant (n=9) , 30±4 ms (Vh = -60 mV). We demonstrate that Dbx1 is a useful marker for XII inspiratory preMNs. This discovery will facilitate further characterization of this previously elusive population of interneurons, including transmitter phenotype and modulatory properties of respiratory pattern-forming neurons.

Disclosures: **A.L. Revill:** None. **A. Kottick:** None. **V.T. Akins:** None. **N.C. Vann:** None. **P.A. Gray:** None. **C.A. Del Negro:** None. **G.D. Funk:** None.

Nanosymposium

204. Language: Spoken and Written

Location: 150B

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 204.01

Topic: F.01. Human Cognition and Behavior

Support: NSF BCS1125756

Title: Processing body-part metaphors recruits the left extrastriate body area (EBA)

Authors: *K. SATHIAN[1], S. LACEY[1], R. STILLA[1], K. MCCORMICK[2], M. BHUSHAN[1], D. KEMMERER[3];
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Abstract: Conceptual metaphor theory (CMT; Lakoff & Johnson, 'Metaphors We Live By', 1980) suggests that knowledge is structured into concepts by metaphorical mapping from sensorimotor experience. CMT therefore predicts that linguistic metaphors should engage brain

regions that mediate sensorimotor experience of the source domain of a metaphor. We provided empirical support for this prediction in a prior event-related functional magnetic resonance imaging (fMRI) study by showing that sentences containing textural metaphors, compared to control sentences matched for meaning, activated somatosensory texture-selective cortex in the parietal operculum (Lacey et al., Brain Lang, 120:416-421, 2012). Here, we used event-related fMRI to investigate whether body-part metaphors would activate neocortical regions housing body-part representations in motor, somatosensory or visual cortex. We constructed sentences employing metaphorical or literal references to the head/face, arm/hand, and leg/foot, and control sentences matched for syllable number and word frequency. Audio recordings of the metaphorical and literal and sentences were matched to their respective controls for speech rate, pitch and intensity. Metaphorical sentences were also matched to their controls for meaning. Participants listened to the sentences while making valence judgments on them, to ensure deep semantic processing. Localizer scans derived from separate scan sessions with the same participants were used to identify parts of left primary motor cortex active during contralateral movements of the lower face, fingers and foot; parts of left postcentral and opercular somatosensory cortex responsive to brushing the contralateral cheek, palm and sole; and visual cortical regions selective for images of faces, arms and legs relative to those of household objects. Within these body-part representations in sensorimotor cortical regions, we found preferential activation for metaphorical relative to control sentences in a region of left extrastriate visual cortex that exhibited limb-selectivity for visual images: the extrastriate body area (EBA). In contrast, sentences containing literal references to body parts did not activate this region preferentially, relative to control sentences. This study, together with our previous observations of texture metaphors, suggests that domain-specific recruitment of sensory cortical regions is a general characteristic of the neural processing of metaphors that have sensory referents, thus offering additional support for CMT.

Disclosures: **K. Sathian:** None. **S. Lacey:** None. **R. Stilla:** None. **K. McCormick:** None. **M. Bhushan:** None. **D. Kemmerer:** None.

Nanosymposium

204. Language: Spoken and Written

Location: 150B

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 204.02

Topic: F.01. Human Cognition and Behavior

Title: Activity in left anterior temporal cortex is modulated by constituent structure of sentences, but only with social/emotional content

Authors: *M. S. MELLEM, K. JASMIN, C. PENG, A. MARTIN;
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Abstract: Both social/emotional processing (e.g., Simmons et al., 2010; Olsen et al., 2007) and building of words into phrases and sentences (i.e., constituent structure; Pallier et al., 2011) have been found to activate anterior areas of the temporal lobe. Often these studies have examined phrase-building processing using sentences of a social and/or emotional nature. This raises the question of whether these phrase-building effects in anterior temporal lobe reflect domain-general effects (for all types of content) or are preferably for social and/or emotional sentences and phrases. To investigate this question we modulated syntactic complexity and content type in a 3x4 design. Subjects were presented with trials consisting of scrambled words, 3-word constituent phrases, or 6-word sentences of 4 content types: Social-Emotional, Social, Object, and Jabberwocky. A trial consisted of six words, each presented for 300 ms (total trial time was 1800 ms): six single words, two 3-word phrases, or one 6-word sentence. Stimuli were matched for total word length, frequency, and concreteness. Forty trials of each condition were presented in a fast event-related design optimally randomized and jittered with the program Optseq2. Subjects were told to silently read the words and respond to the occasional trial instructing them to press a button. Data was acquired on a 7 Tesla Siemens scanner with 1.6 mm isotropic voxels and a TR of 2 seconds. After standard preprocessing and general linear modeling in AFNI, a 2-way ANOVA revealed main effects of Content and Complexity as well as their interaction. Preliminary analyses (n = 9) revealed that the left anterior superior temporal sulcus and gyrus showed a main effect of Complexity ($p<0.01$), and this activity was limited to the Social-Emotional and Social conditions (Content X Complexity interaction; $p<0.01$). Both main effects also overlapped in the left fusiform gyrus. But this area preferred objects (Object > Social-Emotional; $p<0.05$) and showed a Complexity effect for the Object conditions (Object 6-word > Object 1-word; $p<0.09$). In contrast, the triangularis portion of left inferior frontal gyrus (LIFG) was not modulated by content and showed only a main effect of Complexity ($p<0.01$). Thus, whereas LIFG is involved in domain-general syntactic processing, this process is preferentially linked to social and social-emotional stimuli in left anterior temporal cortex. These dissociations challenge the prevailing claims in the field that the anterior temporal lobe is a general phrase-building area. Instead, phrase-building seems to happen within areas that process domain-specific knowledge.

Disclosures: **M.S. Mellem:** None. **K. Jasmin:** None. **C. Peng:** None. **A. Martin:** None.

Nanosymposium

204. Language: Spoken and Written

Location: 150B

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 204.03

Topic: F.01. Human Cognition and Behavior

Support: NIH AG017586

NIH AG032953

NIH AG038490

NIH NS044266,

NIH NS053488

NIH AG00255

Wyncote Foundation

Title: Modulating conceptual combination using focal non-invasive brain stimulation

Authors: *A. R. PRICE[1], M. F. BONNER[1], J. PEELLE[2], M. GROSSMAN[1];
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Abstract: Human thought and language rely on the brain's ability to dynamically construct an unlimited number of complex concepts from a limited set of simpler constituents. For example, individual concepts like “plaid” and “jacket” can be combined into the more complex representation “plaid jacket.” The angular gyrus is a multimodal cortical association area that has been hypothesized to function as an integrative hub in semantic memory. We previously found in both fMRI and patient experiments that the angular gyrus is critical for processing lexical-semantic combinations (e.g., integrating “plaid” and “jacket” into a coherent understanding of “plaid jacket”). In this study, we tested whether we could modulate the integration of semantic information by applying a focal version of anodal transcranial direct-current stimulation (tDCS) to an fMRI-guided region of interest in the left angular gyrus. This technique has been shown to modulate neural excitability by altering resting membrane potential. While traditional applications of tDCS affect broad regions of cortex with poor spatial resolution, high-definition tDCS allows us to apply relatively focal current stimulation by using a ringed array of compact scalp electrodes centered on our cortical region of interest. Participants viewed a pair of words on the screen and indicated by button press whether or not the word pair combined into a meaningful concept (e.g., meaningful combinations like “rusty fence” versus non-meaningful combinations like “steel salad”). The meaningfulness of word pair combinations was determined in a separate norming study, and the word pairs were balanced on a number of psycholinguistic

variables. We tested the prediction that focal current stimulation of the left angular gyrus would enhance the processing of meaningful relative to non-meaningful lexical combinations in healthy adults. We used transcranial current modeling to determine an electrode configuration that would optimally stimulate a region of the left angular gyrus that showed heightened BOLD activity for combinatorial processing in a separate fMRI study. Each participant received a real stimulation condition and an active sham condition. We found that performance on meaningful relative to non-meaningful word pairs was specifically modulated by the application of anodal tDCS over the left angular gyrus. These findings are consistent with the hypothesis that the angular gyrus supports a critical mechanism for integrating coherent lexical combinations, and it appears that this mechanism can be altered by focal tDCS.

Disclosures: **A.R. Price:** None. **M.F. Bonner:** None. **J. Peelle:** None. **M. Grossman:** None.

Nanosymposium

204. Language: Spoken and Written

Location: 150B

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 204.04

Topic: F.01. Human Cognition and Behavior

Support: Wellcome Trust

Medical Research Council (UK)

Title: Predictive mechanisms and speech perception in progressive non-fluent aphasia (PNFA); a magnetoencephalography (MEG) study

Authors: ***T. E. COPE**[1], K. PATTERSON[1,3], E. SOHOGLU[4], C. DAWSON[2], M. GRUBE[5], M. DAVIS[3], J. ROWE[1,3];
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Abstract: Progressive Non-Fluent Aphasia (PNFA) is an adult onset neurodegenerative condition characterised by apraxia of speech and/or agrammatism (Gorno-Tempini et al., 2011). Object knowledge and comprehension of single words is spared, but many patients complain that perceiving speech is effortful, even in optimal listening environments. PNFA typically leads to subtle neuroimaging changes, but changes in cortical thickness in inferior frontal gyrus correlate

with grammatical processing, while those in inferior frontal sulcus correlate with fluency (Rogalski et al., 2011). In normal individuals, activity in these areas is modulated by the congruency of prior knowledge with incoming degraded speech, implying a role in predictive mechanisms that integrate sensory information with prior expectations (Sohoglu et al., 2012). Furthermore, deficits in basic auditory processing have recently been documented in individual patients with PNFA (Vandenberghe et al., 2012). These impairments are typically subclinical but may manifest as difficulty in understanding features of speech prosody (Fletcher et al., 2013). This study assessed the relative contribution of top-down and bottom-up processes to the anecdotal complaint of speech perception difficulties. Eleven patients performed a battery of tests assessing: 1) sensitivity to pitch changes, frequency modulation, and differences in spectro-temporal modulation; 2) the influence of prior stimulus knowledge on the perceived clarity of degraded (noise vocoded) speech; 3) ability to report degraded speech; and 4) ability to discriminate small differences between spoken words. Component 2 was performed with concurrent magnetoencephalography (Elekta Neuromag 306) and 70-channel electroencephalography. Compared to age-matched controls, patients with PNFA demonstrated significant but diverse deficits in basic auditory processing, and were much more affected by the congruency of prior knowledge when rating speech clarity. These results inform our understanding of this frequently reported yet poorly understood symptom in PNFA, and have more general implications for the role of the left frontal lobe in predictive models of speech perception.

Disclosures: **T.E. Cope:** None. **K. Patterson:** None. **E. Sohoglu:** None. **C. Dawson:** None. **M. Grube:** None. **M. Davis:** None. **J. Rowe:** None.

Nanosymposium

204. Language: Spoken and Written

Location: 150B

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 204.05

Topic: F.01. Human Cognition and Behavior

Support: SAO-FRMA grant 09013

FWO G.0660.09

KU Leuven OT/08/056

FWO senior clinical investigator grants: RV, KVL

FWO postdoctoral fellowship: NN

FWO doctoral fellowship: KA

Title: Functional response of the language system to increased amyloid load in cognitively intact older adults

Authors: *K. ADAMCZUK[1,3], A.-S. DE WEER[1,3], N. NELISSEN[1,3], P. DUPONT[1,3], K. VAN LAERE[2,3], R. VANDENBERGHE[1,4,3];
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Abstract: Background and objectives: Word finding symptoms are frequent early in the course of Alzheimer's disease and relate principally to functional changes in the left posterior superior temporal sulcus (Vandenbulcke et al., 2007; Nelissen et al., 2007). We examined how amyloid load affects the network for language and associative-semantic processing in cognitively intact older adults. Methods: Fifty-seven community-recruited cognitively intact subjects, between 52 and 74 years of age, received a detailed neurolinguistic assessment, 18F-flutemetamol PET, and a task-related functional MRI. The fMRI design was factorial with two factors: task (associative-semantic versus visuoperceptual judgment) and input-modality (written words versus pictures). We calculated the standardized uptake value ratio (SUVR) with cerebellar gray matter as a reference region. Our primary outcome analysis consisted of a whole-brain voxelwise linear regression with SUVR in the composite cortical volume of interest (SUVRcomp) as independent variable and fMRI response during associative-semantic versus visuoperceptual processing as dependent variable. The statistical threshold was set at an uncorrected $P < 0.001$ (voxel level) combined with a FWE corrected $P < 0.05$ (cluster level). Results: fMRI response amplitude in the left posterior middle temporal gyrus (cluster peak -57, -45, 9, 64 voxels, cluster level corrected $P = 0.006$) correlated positively with SUVRcomp. This effect was localized to the posterior temporal region that is conjointly activated during semantic processing of words and pictures. The correlation between response amplitude and amyloid load was mainly driven by the contrast between the word conditions (peak cluster -60, -48, 9, 49 voxels, cluster level corrected $P = 0.02$) but not for pictures. Naming reaction times on confrontation naming test correlated with amyloid levels ($r = 0.27$, $P = 0.04$). Analysis of partial volume corrected data confirmed these results. Conclusion: Left posterior middle temporal gyrus, a well-known amodal semantic processing area, undergoes functional changes due to cerebral Aβ related amyloidosis even before clinical symptoms appear. This strengthens the evidence for a critical role of posterior middle temporal gyrus in AD-related language changes. Increased activity with higher amyloid load in cognitively intact individuals together with decreased activity in MCI and early AD is reminiscent of the activity pattern seen in the medial temporal cortex in episodic memory (Sperling, 2007; Reiman et al., 2012) across the different stages of AD.

Disclosures: **K. Adamczuk:** None. **A. De Weer:** None. **N. Nelissen:** None. **P. Dupont:** A. Employment/Salary (full or part-time):; KU Leuven. **R. Vandenberghe:** A. Employment/Salary (full or part-time):; KU Leuven. **K. Van Laere:** A. Employment/Salary (full or part-time):; KU Leuven.

Nanosymposium

204. Language: Spoken and Written

Location: 150B

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 204.06

Topic: F.01. Human Cognition and Behavior

Support: The Spanish Ministry of Economy and Competitiveness RYC-2011-08433

The Spanish Ministry of Economy and Competitiveness PSI2010-19767

Title: Neural correlates of response latencies in the picture naming task

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Abstract: The picture naming task is commonly used to study the neural bases of language production. In a typical experiment, it takes around 700 ms to name a picture. This 700 ms is thought to reflect a composite of neural activities related to the visual recognition of the picture, retrieval of the picture name, and articulation of the picture name. Finding the neural correlates of these picture naming components usually involves manipulating factors assumed to index a given component. Here we took a different approach. We examined how the natural variation in the response latencies in a simple picture naming experiment was determined by changes in the underlying neural activity. To this end, we used the high temporal precision of EEG in combination with a novel statistical technique. In the experiment, participants (N=30) named 100 pictures while their response latencies (mean naming latency = 760 ms, sd = 181 ms) and EEG amplitudes were recorded. The EEG data were analyzed using a novel statistical technique called mixed effect modeling. A major advantage of this regression technique is that it enables the analyses of variables at the single-trial level. Specifically, we examined how the natural variation of naming latencies across individual trials was determined by changes in the EEG amplitudes on these trials. In the absence of a-priori expectations, we conducted a global analysis in which the effect of response latency on EEG amplitude was considered at all time points, for all electrodes.

Protection against multiple comparisons was based on Guthrie and Buchwald (1991). Further, the EEG analysis was based on naming latencies between 0.25 SD above or below the mean naming latency in the experiment. Our results revealed that variation in response latencies was determined by neural activity around 100 ms, and by a larger activity between 400 and 800 ms post-picture onset. Surprisingly, between 100 and 400 ms, neural activity was uncorrelated with naming latencies. These data suggests that with the particular picture naming task used here, the main neural generators of naming latencies arise early around 100 ms, and late between 400 and 800 ms post picture-onset.

Disclosures: **N. Janssen:** None. **J.A. Hernández-Cabrera:** None. **H.A. Barber:** None.

Nanosymposium

204. Language: Spoken and Written

Location: 150B

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 204.07

Topic: F.01. Human Cognition and Behavior

Support: NIH AG017586

NIH NS044266

NIH AG032953

NIH AG038490

Title: Longitudinal decline in sentence comprehension in primary progressive aphasia and behavioral variant frontotemporal degeneration

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Abstract: Patients with the non-fluent/agrammatic variant of primary progressive aphasia (naPPA) have non-fluent speech, grammatical difficulty in expression, and impaired grammatical comprehension. Patients with the logopenic variant of PPA (lvPPA) may overlap in part with naPPA since they also have reduced speech fluency, although we are unaware of comparative studies of grammatical comprehension lvPPA and naPPA. Non-aphasic patients with a behavioral variant of frontotemporal degeneration (bvFTD) also have language difficulties. We

examined profiles of declining grammatical comprehension in non-demented (MMSE>25) patients with naPPA (n=5), lvPPA (n=9) and bvFTD (n=10), diagnosed according to published criteria, and healthy controls (n=22). Patients were assessed twice, separated on average by 20.2 months. We assessed grammatical comprehension with a two-alternative, forced-choice, sentence picture-matching task. Sentences had two semantically-unbiased actors interacting with a reciprocal action, and pairs of pictures associated with each sentence exchanged the roles of the actors. Sentences had a cleft or a center-embedded structure, were subject-relative or object-relative, and included a three-word prepositional phrase between the subject of the main clause and the trace in the subordinate clause or outside this gap. We also assessed, digit span forward and reverse, Boston naming test, and category naming fluency. At baseline, overall comprehension performance differed between groups ($F[3,45]=11.34$; $p<0.001$). Both naPPA and lvPPA differed significantly from controls ($p<0.001$). At the second assessment, all groups - including bvFTD - differed significantly from controls ($p<0.001$). A correlation of the difference score between Time 1 and Time 2 grammatical comprehension assessments with neuropsychological performance found that bvFTD patients' decline correlated ($p<0.01$) with digit span forward and reverse. Decline in bvFTD thus was related to limited short-term memory resources, consistent with our previous cross-sectional findings, but decline in PPA was related to progressive grammatical processing difficulty. We conclude that progressive disease compromises multiple aspects of a large-scale language processing system, leading to declining sentence comprehension over time.

Disclosures: **M. Grossman:** A. Employment/Salary (full or part-time):; University of Pennsylvania. **E. Moran:** None. **D. Charles:** None. **S. Ash:** None. **K. Rascovsky:** None. **C.T. McMillan:** None.

Nanosymposium

204. Language: Spoken and Written

Location: 150B

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 204.08

Topic: F.01. Human Cognition and Behavior

Title: EEG studies of multi-language comprehension

Authors: *T. MAHMOOD[1], Q. MENG[1], P. ACHARJEE[1], E. HONG[2], F.-S. CHOA[1]; [1]UMBC, Baltimore, MD; [2]Univ. of Maryland Sch. of Med. and Maryland Psychiatric Res. Ctr., Baltimore, MD

Abstract: Processing and comprehension of language relies on large scale neuronal synchronization which can be studied through the coherence analysis of electroencephalogram (EEG) signals observed at different locations on the cortex. In this work, we showed that coherence coefficients decreased significantly (>0.3) for certain areas on the cortex when subjects were listening to a word from a language, in which they (a) were not verbally fluent, but could comprehend and (b) they were completely inarticulate along with comprehension. We compared the coherence coefficient measurements with those of their first languages. We also studied and compared results with the first and second languages. Interestingly no obvious coherence coefficient difference could be observed between the comprehension of their first and second languages (verbally fluent with adequate comprehension >10 yrs). A 16-channel EEG system was used to record brain signal from subjects, while they were concentrating on auditory signals, which contained words from different languages, and each wording set was played for 90 seconds. Coherence coefficients between any two channels were calculated using EEGLAB. It was found that differences of coherence coefficients of corresponding channels between the first and second languages of these subjects were all within the range of +0.2/-0.2. On the contrary, for cases (a) and (b), channels on parietal region demonstrated a sharp decrease (> 0.4) in coherence between each other, but coherence coefficients between channel C4 and T4, T6 increased significantly (>0.3,case (a)). Considering different performance of subjects when they were exposed to words of different languages, these results indicated that parietal region played certain role of organizing words during comprehension and finally extended it to verbal articulation. In addition, for the case (a), a global image would be generated in the brain to help comprehension and memorization and this activity could be detected on the right side of the brain. From frequency domain spectrum, it could also be observed that the highest values of coherence coefficient were always within the frequency range of 10 Hz to 12 Hz.

Disclosures: **T. Mahmood:** None. **Q. Meng:** None. **P. Acharjee:** None. **E. Hong:** None. **F. Choa:** None.

Nanosymposium

204. Language: Spoken and Written

Location: 150B

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 204.09

Topic: F.01. Human Cognition and Behavior

Support: NSF SMA-0835976

Title: Localizing categorical speech representations in perception and production

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Abstract: Human speech is subserved by a bilateral network of cortical regions, including superior temporal, inferior parietal and inferior frontal cortex, and utilizes auditory, phonological, and motor representations of speech sounds. The precise nature of these representations is difficult to assess due to the limitations of functional neuroimaging and the complexity of speech sounds, for which suitable controls are difficult to construct. Multi-voxel pattern analysis (MVPA) provides a tool for studying representations using the accuracy of statistical models as indicators of local information content. In this fMRI study, 13 healthy, adult English speakers were presented with systematically varying consonant-vowel-consonant syllables in a delayed repetition task. Each trial began with the auditory presentation of a syllable, and overt repetition was visually cued after a ~9s delay. Two whole-brain EPI volumes were collected per trial, timed to the peak hemodynamic responses to the stimulus onset and the production cue. Cortical surfaces were reconstructed and MVPA, using a surface-based searchlight approach, was used to generate information maps for discrete stimulus features based on the cross-validation accuracy of linear support vector machines. Maps were created for the onset, vowel and coda of each syllable and for syllable identity, separately based on the perception and production responses. Group-level results show left-lateralized regions in the posterior superior temporal sulcus (STS) and ventral inferior frontal gyrus (IFG) that significantly predicted vowel identity for perception events. For production events, several STS clusters were identified, along with a more dorsal IFG region at the junction of the inferior frontal sulcus; further, vowel information was strongly left-lateralized for production. Consonant information in production was mainly localized to bilateral sensorimotor cortex, suggesting a basis in articulatory planning or somatosensory feedback. Prediction of whole syllables highlighted a network of temporal, parietal, and frontal regions. These syllable-level maps were compared to prediction accuracies from constituent phonemes to determine regional preferences for phonemic vs. whole-syllable information. These results help to inform models of speech and phonological working memory and demonstrate methods for assessing representations of complex stimuli with coarse measurement techniques. This approach has implications for brain computer interfaces which depend on native, task-relevant representations rather than employing generic decision-making strategies with task-specific software.

Disclosures: **C.J. Johnson:** None. **J.W. Bohland:** None.

Nanosymposium

204. Language: Spoken and Written

Location: 150B

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 204.10

Topic: F.01. Human Cognition and Behavior

Support: Doris Duke Charitable Foundation Grant #2012062

Title: Interhemispheric frontal resting connectivity increases in post-stroke aphasia and is associated with worse performance

Authors: *L. M. SKIPPER[1], E. H. LACEY[1,2], S. XING[1], A. DESKO[1], M. FAMA[1], X. JIANG[1], P. TURKELTAUB[1,2];
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Abstract: The role of the right hemisphere in aphasia recovery has been debated for over a century. Some argue that the right hemisphere plays a compensatory role, aiding recovery (e.g., Basso et al., 1989), while others posit that right hemisphere activity interferes with recovery (e.g., Barwood et al., 2011). Recently, research has shifted to examining connectivity, rather than activation levels, in order to better understand neural patterns that explain aphasia symptoms and recovery (Bonilha et al., 2014). This approach is critical because some differences in task-related activity in aphasia may relate to differences in the effort required for task performance rather than actual reorganization of language networks. This experiment examined the resting state networks associated with right BA 44 and performance on a range of language tasks. Twenty participants with left hemisphere lesions and aphasia diagnoses, as well as 21 age matched controls, participated in this study. The participants underwent a 7 minute T2* weighted resting state MRI scan, as well as a high-resolution structural scan. Participants also underwent a battery of language and other cognitive tests. The time course in right BA 44 was extracted for each participant. The model used the time course in right BA 44 as the predictor, and included motion parameters as covariates. At the group level, participants with aphasia showed greater connectivity to right BA 44 in the left and right middle temporal gyrus, right inferior temporal lobe and left insula, compared to controls. The peak difference in connectivity was in the left insula. Participants in the aphasia group were then grouped based on whether each individual's lesion overlapped with this peak (10 in each group). Participants with lesions overlapping with the peak showed significantly impaired performance on a range of generative naming and tasks and tasks that required reading words aloud, relative to patients whose lesions did not overlap with the left insula, and controlling for lesion size. Finally, connectivity between the left insula and right BA 44 was measured for each aphasic participant who had a preserved left insula. Connectivity in this group correlated negatively with performance on three different oral word reading tasks and two tests of speech apraxia, controlling for lesion size. These results

demonstrate that connectivity between left and right frontal lobes increases after a left hemisphere stroke, and that this over-connectivity is related to worse performance on certain speech measures. Additional analyses will be needed to determine if similar connectivity relationships are observed with other areas of the right hemisphere.

Disclosures: **L.M. Skipper:** None. **E.H. Lacey:** None. **S. Xing:** None. **A. Desko:** None. **M. Fama:** None. **X. Jiang:** None. **P. Turkeltaub:** None.

Nanosymposium

204. Language: Spoken and Written

Location: 150B

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 204.11

Topic: F.01. Human Cognition and Behavior

Support: ANR-10-BLAN-1403

ANR-10-IAIHU-06

Title: Music reading in the brain: How musical literacy shapes the ventral cortical visual stream

Authors: V. MONGELLI[1,2,3], *P. BARTOLOMEO[4,5,3,6,2], F. VINCKIER[1,3,2], S. DEHAENE[7,8], I. PERETZ[9,10,11], L. COHEN[1,3,6,2];
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Abstract: The visual word form area (VWFA), situated in the left fusiform gyrus of the human brain, plays an important role in the visual identification of letters and their order (Cohen et al. Brain 2000). Its activity during word reading depends on expertise, being stronger in early literate subjects than in illiterate ones (Dehaene et al. Science 2010). Here, we examine if this region is also important for music reading. Using fMRI with an irrelevant detection task, we measured brain responses to written musical notations in 21 professional musicians and 23 musically naive controls. Music-induced responses were identified and compared to the

responses induced by words, faces, houses, and tools in the VWFA and in other category-selective regions of the ventral cortical visual stream (face fusiform area, FFA; para-hippocampal place area; lateral occipital complex). Results showed that in both groups music induced weaker activations than did the relevant preferred categories (e.g., faces in FFA) in all regions (all Ps<0.009), except for the VWFA, where music-induced responses did not differ from the preferred category (i.e., words; F<1) (interaction of the "music vs preferred" factor x region F(5,210)=24, P<0.001). Zooming in on the VWFA, we observed a difference between groups. In control subjects, music activated the VWFA marginally less than words (F(1,22)=4.06, P=0.056). Conversely, in musicians, VWFA activation was stronger for music than for words (F(1,20)=9.01, P=0.007; interaction group x type of stimulus F(1,42)=12, P=0.0012). Thus, musical literacy changed the functional properties of the VWFA (but not of the other explored regions), by increasing the activation for musical notation over the activation for written words. Complementary analyses demonstrated that musical literacy also modulated the spatial properties of the VWFA. The peaks of the main activations induced by words, faces, houses, and tools were identified for musicians and controls independently of music-induced activations. In a 15-mm radius sphere centered on the peak of the VWFA, the peaks for words were more anterior (F(1,42)=9.05, p=0.0044), more lateral (F(1,42)=7.18, p=0.010), and more dorsal (F(1,42)=14.34, p=0.0005) in musicians than in controls. Thus, we show that music reading may share neural mechanisms used for word reading. Our results are consistent with findings from brain-damaged musicians, who lost their ability to read music and words alike after damage to ventral regions in the left hemisphere. Moreover, we show for the first time that musical literacy modifies the functional and spatial properties of reading-selective regions in the brain.

Disclosures: **V. Mongelli:** None. **P. Bartolomeo:** None. **F. Vinckier:** None. **S. Dehaene:** None. **I. Peretz:** None. **L. Cohen:** None.

Nanosymposium

204. Language: Spoken and Written

Location: 150B

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 204.12

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R00 HD065839

Title: Unexpected neural correlates of the word-nonword distinction in lexical decision

Authors: *W. W. GRAVES, O. BOUKRINA, E. J. ALEXANDER, S. R. SMOLIN, R. LATHIA;
Psychology, Rutgers Univ., Newark, NJ

Abstract: The distinction between words and nonwords is a fundamental one, and its neural correlates have been widely investigated using techniques such as fMRI. When directly contrasted, words typically activate areas associated with processing word meanings (semantics), such as the angular (AG) and posterior cingualte (PC) gyri. Pronounceable letter strings (pseudowords) typically activate areas associated with attention or working memory demands, such as the inferior frontal junction (IFJ) and intraparietal sulcus (IPS), along with the posterior occipito-temporal sulcus (pOTS). The current study used fMRI with 20 healthy, right-handed, typical adult readers and found the reverse of the above pattern. Words activated attention-related areas; pseudowords activated areas previously associated with semantic processing, and supramarginal gyrus (SMG). This was a surprising result, considering that pseudowords do not have meaning. To examine the possibility that this pattern was a result of using word stimuli that were of lower corpus frequency than is typically used, a follow-up experiment (N = 11) was performed in which all the high-frequency words were presented in the first half of the experiment, followed by all the low-frequency words in the last half. Although similar regions were activated in the word-pseudoword (lexicality) contrast in both halves of the experiment, an interaction between word frequency and lexicality was significant in the left IPS, putamen, and bilateral occipital cortex. Comparisons to baseline in the IPS showed similar activation for low-frequency words and pseudowords, both of which were less than high-frequency words. To help determine the causal dynamics behind these differences, we performed Bayesian effective connectivity analysis using the following regions: AG, IFJ, IPS, PC, SMG and pOTS. This revealed top-down influences from IFJ and IPS to pOTS only in the low-frequency condition. These results suggest that individual word characteristics (in this case, word frequency) can influence the neural correlates of a distinction as basic as lexicality to such an extent as to reverse its typical neural signature. A possible mechanism for this reversal is the differential engagement of top-down executive or attentional systems.

Disclosures: W.W. Graves: None. **O. Boukrina:** None. **E.J. Alexander:** None. **S.R. Smolin:** None. **R. Lathia:** None.

Nanosymposium

204. Language: Spoken and Written

Location: 150B

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 204.13

Topic: F.01. Human Cognition and Behavior

Title: Neural specialization for interpersonal communication within dorsolateral prefrontal cortex: A NIRS investigation

Authors: *J. HIRSCH[1,2], J. A. NOAH[1], X. ZHANG[1], S. YAHIL[1], P. LAPBORISUTH[1], M. BIRIOTTI[3];
[1]Dept. of Psychiatry, [2]Dept. of Neurobio., Yale Sch. of Med., New Haven, CT; [3]Med. Humanities, Univ. Col. London, London, United Kingdom

Abstract: Although interpersonal interaction and communication play a fundamental role in human socialization, little is known about the neural correlates that underlie these critical processes. This current knowledge gap is due, in large part, to the lack of techniques and methods to investigate neural responses from two or more interacting individuals. Conventional neuroimaging techniques such as fMRI have focused on principles of neural organization associated with single brains. A goal of this study is to extend the current focus from single brains to understanding neural events that mediate interpersonal communication in two brains. We used near-infrared spectroscopy (NIRS) to simultaneously record brain activity from subject pairs engaged in either interpersonal dialogue or similar monologue conditions. We hypothesized that canonical speech production systems would be differently sensitive to dialogue conditions. NIRS detects BOLD signals from surface optodes that compare absorption spectra indicating relative oxy and deoxy hemoglobin levels. fMRI and NIRS signals have been shown to be highly correlated within the superficial layers of the brain, and NIRS has the advantage of data acquisition during natural interactions between the two subjects. In both the dialogue and monologue conditions, the subjects alternated between talking and listening in 15 second epochs, and the topics were based on objects presented to both S1 and S2 from the classic Boston Naming task. Each “run” lasted for six minutes and consisted of 24 epochs. Optodes were positioned in homologous locations for each of the subject pairs within the left dorsal lateral prefrontal cortex based on the standard MNI human atlas as determined by the Polhemus digitizer for each subject. Block-related averages revealed the expected anti-correlation between subjects during talking and listening with significant ($p<0.008$)) amplitude increases for the dialogue conditions. The S1 to S2 co-variation between residual signals (the hemodynamic response function was removed) was highest for the dialogue condition ($p<0.05$), and wavelet coherence analysis showed stronger episodic synchrony throughout the dialogue condition than in the monologue for these canonical speech regions. These findings are the first to document neural specialization for interpersonal communication based on “dual-brain” measures of synchrony and coherence within homologous dorsal lateral prefrontal cortices of subject pairs, and extend neuroimaging investigations to the dynamical interactions between individuals.

Disclosures: **J. Hirsch:** None. **J.A. Noah:** None. **X. Zhang:** None. **S. Yahil:** None. **P. Lapborisuth:** None. **M. Biriotti:** None.

Nanosymposium

204. Language: Spoken and Written

Location: 150B

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 204.14

Topic: F.01. Human Cognition and Behavior

Support: NIH 2R01DC05660

Title: Cortical dynamics underlying online building of hierarchical structures

Authors: ***N. DING**[1], H. ZHANG[1], X. TIAN[1], L. MELLONI[1,2,3], D. POEPPEL[1];
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Abstract: Language is hierarchically organized into syllables, words, phrases, and sentences. For spoken language, online building of these hierarchical linguistic structures is a fundamental yet challenging task. Although the boundaries between syllables generally have clear acoustic signatures, determining the boundaries between words and phrases critically relies on the listener's linguistic knowledge. During speech listening, it has been well characterized that auditory cortical activity is entrained to the syllabic rhythm of speech. However, how larger linguistic structures, such as words and phrases, are represented in the brain remains elusive and is investigated in this study. We designed speech materials in which the hierarchical linguistic structure of speech is dissociated from low level acoustic features, and measured cortical activity using magnetoencephalography (MEG) from listeners listening to such materials. It is demonstrated that cortical activity is hierarchically entrained to the rhythms of words, phrases, and sentences, unconfounded by the tracking of acoustic properties of speech. Furthermore, such hierarchical entrainment is demonstrated to be associated with the syntactic structure of speech rather than the predictability of each incoming word. In summary, cortical circuits can generate slow rhythms matching the time scales of larger linguistic structures, even when such rhythms are not present in the speech input, which provides a plausible mechanism for online building of large linguistic structures.

Disclosures: **N. Ding:** None. **H. Zhang:** None. **X. Tian:** None. **L. Melloni:** None. **D. Poeppel:** None.

Nanosymposium

205. Working Memory

Location: 147A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 205.01

Topic: F.01. Human Cognition and Behavior

Support: NINDS NS065046

Alfred P Sloan Foundation

James S. McDonnel Foundation

Title: Dissociable effects of frontal cortex stimulation during selection from working memory

Authors: *C. CHATHAM[1], D. BADRE[2];
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Abstract: Working memory refers to the capacity to maintain information in the service of goal-oriented behavior. A variety of evidence suggests that input to working memory is controlled by a gate. This "input gate" selects relevant information for maintenance in working memory while keeping distractors out. However, not all information in working memory will be relevant for guiding behavior at any given moment. For this reason, working memory may also require an output gate. When the output gate is open, the contents of working memory are selectively granted an influence over behavior. This kind of multiply-gated architecture has computational utility, and we have recently shown that output gating in particular is associated with differential BOLD in corticostriatal circuitry (Chatham, Frank & Badre, 2014). However, the necessity of these circuits for output gating cannot be established using fMRI. Here we use TMS to test the causal contribution of the two cortical regions identified with fMRI: the dorsal premotor cortex [PMd] and the dorsal anterior premotor cortex [pre-PMd]. We delivered single pulse TMS to each region during the same hierarchical working memory task used in fMRI. TMS dissociated these two regions, such that PMd stimulation elicited reliable peripheral motor activity (as recorded with simultaneous EMG), whereas pre-PMd stimulation elongated reaction times only under conditions requiring output gating of working memory. Effects of pre-PMd stimulation on output gating were temporally specific, observed only within a 340-410 ms window following the onset of context retrocues. In addition, these effects were observed only when subjects needed to reorient attention away from the most recently presented item. These results demonstrate a causal dissociation between the roles of PMd and the pre-PMd. As such, these results constrain models of fronto-striatal interactions and output gating, and motivate the consideration of attentional dynamics not commonly considered within those frameworks.

Disclosures: **C. Chatham:** None. **D. Badre:** None.

Nanosymposium

205. Working Memory

Location: 147A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 205.02

Topic: F.01. Human Cognition and Behavior

Support: NIMH R01-MH087214

Title: Phase-dependent coding of feature-selective population codes during visual working memory storage

Authors: ***D. E. ANDERSON**[1,2], J. T. SERENCES[3,4], E. K. VOGEL[1,2], E. AWH[1,2];
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Abstract: Oscillatory coding models posit that rhythmic neural activity coordinates feature-selective cellular assemblies coding for stimulus-specific information. During active storage of representations in working memory (WM), oscillatory coding models predict the emergence of online visual representations from sustained patterns of rhythmic feature-selective neural activity. Recently, we employed a forward encoding model of orientation-selectivity to reconstruct orientation-selective channel tuning functions (CTFs) from spatial patterns of induced oscillatory activity during an orientation WM task (Anderson et al., in press, J. Neurosci.). Our work revealed delay-specific orientation-selective activity in the alpha (8-12 Hz) frequency band, and the selectivity of CTFs constructed from this activity predicted both the content and quality of mnemonic representations. Two empirical patterns motivate our hypothesis that synchronized feature-selective neural activity in the alpha band plays a role in the storage of information in visual WM: (1) feature-selective assemblies are locally synchronized in high gamma frequency, and (2) peak amplitudes of gamma activity is phase-locked to alpha phase during WM storage. Examined together, a clear prediction arises from these results. If the functional role of rhythmic alpha activity is to bind feature-selective assemblies into discrete temporal units, then spatial patterns of induced alpha activity should reveal a phase-dependent profile of orientation-selectivity. In experiment 1, we tested this prediction by reconstructing orientation-selective CTFs from phase-binned patterns of induced delay-specific alpha activity during the storage of a single orientation stimulus. Supporting predictions of phase-dependent coding, reliable CTFs emerged during a single phase-band that was centered on 0 degrees. In

experiment 2, we manipulated storage load (while maintaining similar stimulus properties) by presenting two memoranda in temporal succession. Here, phase-coding models predict that individuated representations are maintained by temporally segmented population codes. Consistent with previous work in nonhuman primates (Siegel & Miller, 2009), phase-locked tuning profiles associated with each item were desynchronized, such that the preferred phase angle of the first item was shifted with respect to the preferred phase angle of the second item by approximately 60 degrees. Thus, we provide empirical support for phase coding models of WM storage in humans, and propose that online representations are maintained by phase-dependent coordination of feature-selective population codes.

Disclosures: D.E. Anderson: None. **J.T. Serences:** None. **E.K. Vogel:** None. **E. Awh:** None.

Nanosymposium

205. Working Memory

Location: 147A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 205.03

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01-MH092345 (JTS)

NSF GRFP (TCS)

NIH Grant T32-MH020002-12 (EFE)

Title: Mnemonic representations in human occipital, parietal and frontal cortex index visuospatial working memory acuity

Authors: *T. C. SPRAGUE[1], E. F. ESTER[2], J. T. SERENCES[1,2];
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Abstract: Working memory (WM) enables the maintenance and manipulation of information no longer immediately available in the environment. Typically, as more information is actively maintained in visual WM, behavioral reports about the maintained information decrease in precision. Neural responses associated with the maintenance of information in WM have been found in parietal and frontal cortex (Gnadt & Andersen, 1988; Funahashi et al, 1989; Todd & Marois, 2004; Xu & Chun, 2006). In parallel, human neuroimaging experiments have found that patterns of activation measured with functional magnetic resonance imaging (fMRI) of occipital and posterior parietal cortex, scalp electroencephalogram (EEG) recordings, or

magnetoencephalogram (MEG) recordings reflect the identity of a remembered stimulus (Serences et al, 2009; Harrison & Tong, 2009; Ester et al, 2009; Jerde et al, 2012; Riggall & Postle, 2012; Christophel et al, 2012; Ester et al, 2013; Emrich et al, 2013; Albers et al, 2013; LaRocque et al, 2013; Pratte & Tong, 2014; Anderson et al, 2014). Many models of visual WM hold that the signal-to-noise ratio of these population-level responses should be associated with performance on visual WM tasks, both within participants and across memory load conditions, and across participants (Bays 2014; Ma et al, 2014). We tested the prediction that the fidelity of population-level codes for a stimulus (or stimuli) held in visuospatial WM is associated with behavioral performance by implementing an image reconstruction technique (Sprague & Serences, 2013) to compute multivoxel region-level spatial representations of remembered locations using fMRI data patterns measured during a delay interval. Participants remembered the precise location(s) of 0, 1 or 2 small stimuli over a delay interval during scanning. We observed accurate reconstructions of remembered - but not forgotten - spatial locations using activation patterns from occipital (V1-V3A; hV4), parietal (IPS0-IPS3) and frontal (sPCS) regions of interest. Furthermore, we quantified these reconstructed spatial representations to evaluate whether differences in mnemonic precision both within and across participants are associated with the amplitude of these spatial representations over baseline, their size (precision), or a univariate response increase. Spatial representations were lower in amplitude when set size was increased (and, accordingly, when behavioral performance was degraded), suggesting that the amplitude of reconstructed spatial representations from population codes reflects the fidelity of the covert mnemonic representation which constrains behavioral performance on this task.

Disclosures: **T.C. Sprague:** None. **E.F. Ester:** None. **J.T. Serences:** None.

Nanosymposium

205. Working Memory

Location: 147A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 205.04

Topic: F.01. Human Cognition and Behavior

Support: DFG grant AX82/2

Title: Alpha band activity in the dorsal and ventral stream reflect a double dissociation between feature specific networks during working memory maintenance

Authors: ***M. LESZCZYNSKI**[1], A. JAHANBEKAM[1], J. FELL[1], O. JENSEN[2], N. AXMACHER[1,3];

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Abstract: Successful working memory (WM) requires both maintenance of relevant and inhibition of irrelevant information. The posterior alpha rhythm (8-13Hz) observed over the occipital cortex and posterior parietal cortex has been found to inhibit visuo-spatial and orientation information during WM maintenance. As such it has been suggested that the alpha band activity reflects the allocation of resources by inhibiting and disinhibiting specific regions. While this case has been made for the dorsal stream, it remains elusive if the alpha oscillations play a similar role in the ventral stream. Here we used a delayed match-to-sample task engaging either the ventral or the dorsal visual stream. Depending on the condition, participants were required to memorize the identity or the spatial orientation of a face. Passive viewing with no memory load was used as a control condition. We recorded the intracranial EEG (iEEG) in 13 human epilepsy patients to test if the ventral and dorsal visual streams show feature specific changes in oscillatory brain activity. Using a non-parametric cluster based permutation statistics, we observed a double dissociation: alpha power was reduced in the ventral stream during maintenance of 'face identity' as compared to 'face orientation' and the control condition. In contrast, the power of alpha activity was reduced in the dorsal stream during maintenance of 'face orientation' as compared to 'face identity' and control. These results show that neural oscillations in the alpha band are modulated in feature specific networks in the ventral and dorsal visual stream during working memory maintenance. They suggest that decreases of alpha power contribute to WM by selective disinhibition of areas that represent currently relevant features. Importantly, our intracranial recordings demonstrate that the functional role of alpha band activity observed in visual regions using EEG/MEG generalize to the ventral stream.

Disclosures: **M. Leszczynski:** None. **A. Jahanbekam:** None. **J. Fell:** None. **O. Jensen:** None. **N. Axmacher:** None.

Nanosymposium

205. Working Memory

Location: 147A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 205.05

Topic: F.01. Human Cognition and Behavior

Support: NIH R01 MH064498

Title: Frontoparietal contributions to the short-term retention of motion and color

Authors: *A. C. RIGGALL[1], N. S. ROSE[2], M. J. STARRETT[2], B. R. POSTLE[2];
[1]Psychology, [2]Univ. of Wisconsin-Madison, Madison, WI

Abstract: Given recent empirical findings supporting the sensory recruitment hypothesis, which posits that short-term storage occurs in the same neural systems specialized for initial sensory processing, an important question remains: what functions, if not storage, are the frontoparietal areas that reliably show load-dependent, elevated activity during the delay-period of short-term memory tasks performing, if not storage? One compelling possibility is that these regions are involved in directing top-down attention towards these internal representations in sensory regions to support their maintenance. Under this hypothesis we would expect to see increased involvement of these regions with increased memory load, as additional attentional resources are needed to maintain additional memory items. In the current study we manipulated load in a short-term delayed-recall task that required participants to remember either the direction of motion in multiple sequentially presenting moving dot stimuli, or the color of the dots in each moving dot stimulus (loads 1 and 3, direction or color in separate block). During an initial session we collected BOLD data while participants performed this task in the scanner. These data were then used to identify two load-sensitive frontoparietal regions, the inferior frontal junction (IFJ) and the posterior intraparial sulcus (pIPS), which were subsequently targeted with transcranial magnetic stimulation during a TMS/EEG session, during which participants performed the same delayed-recall task for motion and color. We found differing patterns of functional connectivity in the BOLD data, depending on the specific task condition and load. We observed increases in effective connectivity, as indexed by increases in the strength and spread of the TMS-evoked response, at higher loads while stimulating frontal and parietal regions, but not a visual region (area MT). These results are consistent with a top-down attentional mechanism, controlled by frontoparietal regions, supporting short-term maintenance within "sensory" regions.

Disclosures: **A.C. Riggall:** None. **B.R. Postle:** None. **N.S. Rose:** None. **M.J. Starrett:** None.

Nanosymposium

205. Working Memory

Location: 147A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 205.06

Topic: F.01. Human Cognition and Behavior

Title: Flexibility of representational states in working memory

Authors: *N. ZOKAEI[1], S. MANOHAR[1], S. NING[1], E. FEREDOES[2], M. HUSAIN[1];
[1]Oxford Univ., Oxford, United Kingdom; [2]Reading university, Reading, United Kingdom

Abstract: Recent research has shown that the relationship between working memory (WM) and attention is a highly interdependent one, with evidence that attention determines the state in which items in WM are retained. Through the focusing of attention, an item might be held in a more prioritized state, commonly termed the focus of attention (FOA). Items outside the FOA, although still retrievable, are considered to be in a different representational state. Although much research has focused on the representational qualities of the item in FOA, less is known on the nature of the other (non-FOA) items in WM. We present data from a series of experiments that used various methods of manipulating WM representational states to examine outstanding questions on the nature, mechanisms and neural instantiation of these states. One means for bringing an item into FOA is to use 'retro-cues' to direct attention to the most relevant item in memory. Alternatively, an item can enter the FOA through bottom-up mechanisms (e.g. sequential presentation of items with final item being in FOA) or by performing an action on one of the retained items ('incidental' cueing). In all these cases, the item in FOA is recalled with greater precision compared to other WM items. Across two experiments, using combinations of incidental cues and sequential item presentation, we demonstrate that when an item remains behaviourally relevant, despite not being initially inside the FOA, focusing attention upon it can increase its recall precision. This would suggest for items outside the FOA information about them can still be retrieved and they can be flexibly shifted into the FOA. In contrast, a retro-cueing approach demonstrated that once an item is rendered behaviourally unimportant, it cannot be brought into the FOA, nor recalled with higher precision. We used transcranial magnetic stimulation (TMS) over early visual cortex to disrupt visual stimuli held in WM. Only items in FOA were affected by TMS, with no change to recall precision of the other items. These results provide direct neural evidence for at least two different representational states in WM. Taken together, our results support different representational states in WM in which the flexible shifting of information between them depends on the methodology used. The item in FOA was represented with higher precision across all experiments, but the recall precision of other items is crucially dependent on the relevance of these items to the WM task. These findings have important consequences for emerging state-dependent models of WM, with the movement between states being flexible under some conditions and non-existent under others.

Disclosures: **N. Zokaei:** None. **S. Manohar:** None. **S. Ning:** None. **E. Feredoes:** None. **M. Husain:** None.

Nanosymposium

205. Working Memory

Location: 147A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 205.07

Topic: F.01. Human Cognition and Behavior

Support: Research supported by National Institute for Health Research (NIHR) Oxford Biomedical Research Centre based at Oxford University Hospitals Trust Oxford University

Title: Top-down control of working memory in ageing: A magnetoencephalography study

Authors: ***R. M. MOK**, N. E. MYERS, G. WALLIS, A. C. NOBRE;
Univ. of Oxford, Oxford, United Kingdom

Abstract: Working memory (WM) abilities decline as we age. Studies in younger adults have shown that top-down attention can be used to improve WM performance. However, the ability to control attention to WM representations and the neural mechanisms that underlie these abilities in ageing have not previously been studied. To study the neural dynamics of WM in ageing, a large sample (N=75) of older adults (60 years+) performed a precision WM task with or without an attention-guiding cue whilst undergoing a magnetoencephalography (MEG) scan. Attention-directing cues presented in the maintenance period of the trial (retro-cues) conferred a significant advantage for WM performance, suggesting preserved attentional mechanisms in older adults. Low frequency oscillations in the alpha band (8-12Hz) during the delay period after a retro-cue indexed attentional orienting to the cued item location. Specifically, comparing trials with right versus left retro cues revealed decreased alpha synchronization lateralized at left parietal and visual sensors and increased alpha synchronization at right parietal and visual sensors. Investigating the neural mechanisms related specifically to orienting attention in WM (retro-cue minus neutral cue) revealed left lateralized desynchronization in low frequency oscillations, potentially arising from the left parietal cortex. Preliminary results indicate that synchronization of high frequency oscillations (> 60Hz) accompanies the desynchronization in lower frequencies. Notably, these attentional effects are punctate, wherein the strongest activations appear to occur immediately after the cue and slowly return to baseline. These data suggest that intact WM control mechanisms may correspond to similar attentional mechanisms in younger adults.

Disclosures: **R.M. Mok:** Other; Studentship support paid by principle investigator's grant, supported by the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre. **N.E. Myers:** None. **G. Wallis:** None. **A.C. Nobre:** None.

Nanosymposium

205. Working Memory

Location: 147A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 205.08

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant EY021644

NIH Grant EY022229

Title: Divergence and convergence of attention network activity in visual attention and short-term memory

Authors: *S. L. SHEREMATA[1], B. CARTER[1], D. C. SOMERS[2], S. SHOMSTEIN[1]; [1]Psychology, George Washington Univ., Washington, DC; [2]Psychology, Boston Univ., Boston, MA

Abstract: Within the dorsal attention network, areas of the intraparietal sulcus (IPS) and the frontal eye fields (FEF) have been implicated in both visual attention and short-term memory (VSTM). As these related tasks share a number of cognitive processes, such as spatial selection and indexing, it is unclear to what extent activity reflects the same underlying neural mechanisms. Using fMRI, we employed attention and VSTM tasks with identical stimuli and similar selection and indexing demands to ask whether signal amplitude and contralateral bias differentiated processing in IPS1/2 and FEF, a putative sub-network of the dorsal attention network, as well as how they interacted with regions of ventral parietal and visual cortex. While memory and attention largely engaged similar regions, BOLD activity in IPS1/2 was overall greater for VSTM than for attention. Furthermore, consistent with its role in distractor suppression during memory, the right angular gyrus, an area of the ventral parietal cortex, showed greater deactivation during memory than attention. Interestingly, there was no significant difference in FEF, demonstrating that parietal areas of the dorsal attention network selectively disassociated attention and memory. These results demonstrate a divergence between the two tasks within retinotopic and ventral parietal cortex, suggesting that memory engaged additional processes in these areas. In terms of spatial bias, however, attention and memory showed similar patterns. Across the sub-network, activity was greater for items presented in the contralateral, or opposite, hemifield. Furthermore, consistent with previous findings, there was a stronger contralateral bias in the left than the right hemisphere across both tasks. Our findings show that processes underlying both VSTM and attention demonstrate spatial biases in an asymmetric manner across the hemispheres and suggest that these asymmetries are characteristic of this sub-

network across the two cognitive tasks. Finally, we measured the degree of functional connectivity across areas of the dorsal attention network, ventral parietal cortex, and occipital lobe during each task to determine to what degree these networks interact in a task-dependent manner. Taken together, our results demonstrate that parietal activity distinguishes between memory and attention, lending evidence for specialized memory processes in the parietal cortex.

Disclosures: S.L. Sheremata: None. **B. Carter:** None. **D.C. Somers:** None. **S. Shomstein:** None.

Nanosymposium

205. Working Memory

Location: 147A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 205.09

Topic: F.01. Human Cognition and Behavior

Support: NIH grant F32EY022874 to KCB

NIH grant 1R01EY022355 to YX

Title: Decoding under distraction reveals distinct occipital and parietal contributions to visual short-term memory representation

Authors: *K. BETTENCOURT, Y. XU;
Vision Lab., Harvard Univ., Cambridge, MA

Abstract: Recently, there has been considerable debate on where the contents of visual short-term memory (VSTM) are stored. Univariate fMRI analyses have suggested that regions in the parietal lobe, in particular superior intraparietal sulcus (IPS), play a central role in VSTM storage. In contrast, fMRI multivariate pattern analysis (MVPA), has primarily implicated sensory cortices, in particular early visual cortex, in the storage of visual information. These findings prompt two important questions: 1) what does the dissociation between these techniques tell us about how VSTM is stored in the brain, and 2) if early visual areas, whose main role is to process incoming visual stimuli, are involved in the storage of VSTM, what happens to that stored information when subsequent visual information must be processed? We examined these questions across three fMRI studies using MVPA. We found that, while behavioral performance was not affected by the presence of distracting visual stimuli during the delay, in early visual cortex, decoding performance depended, not only on whether distractors were present during the

delay, but also on participants' foreknowledge of their presence. When participants knew distractors would be present, decoding performance for the remembered grating fell to chance. This lack of decoding in early visual cortex was not due to a failure of fMRI MVPA to resolve a weak memory signal amongst a stronger distractor signal, as the same distractor signal had no impact on decoding of a similarly weak grating stimuli presented perceptually. Instead, we found that decoding accuracy rose as the probability of distraction decreased, suggesting a switch in participants' strategy based on distractor predictability. However, in superior IPS, supporting previous univariate findings, the remembered grating could be successfully decoded, regardless of the presence, absence, or predictability of distractors during the delay. This finding was specific to superior IPS, as other parietal regions, including topographically defined IPS regions, showed inconsistent decoding across distractor presence and predictability. These results bring together the univariate and MVPA literature and demonstrate that superior IPS plays a central role in VSTM information storage. Early visual areas, however, are unlikely to be essential for memory storage, and may reflect cognitive processes, such as visual rehearsal or imagery, that can be brought online, particularly under low visual processing loads.

Disclosures: K. Bettencourt: None. **Y. Xu:** None.

Nanosymposium

205. Working Memory

Location: 147A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 205.10

Topic: F.01. Human Cognition and Behavior

Support: NWO VICI Grant 453-09-002

Title: Frontoparietal structural connectivity predicts ability to top-down modulate alpha and gamma oscillations in visual areas

Authors: *T. R. MARSHALL[1], T. O. BERGMANN[1,2], O. JENSEN[1];
[1]Donders Inst., Nijmegen, Netherlands; [2]Inst. of Psychology, Christian Albrechts Univ., Kiel, Germany

Abstract: Directing covert attention produces retinotopically-specific, behaviourally relevant modulations of visual cortical oscillations. Alpha (8-12Hz) oscillations increase in regions processing unattended input whereas gamma (50-90Hz) oscillations increase in regions processing attended parts of a visual scene. The dorsal attentional network (DAN) - in particular

the frontal eye field (FEF) - may provide top-down control of these modulations, and hemispheric asymmetries in the superior longitudinal fasciculi (SLF) believed to connect FEF to posterior cortex have been shown to predict behavioural attentional biases. We combined magnetoencephalography (MEG) with high angular resolution diffusion imaging (HARDI) in order to investigate the structural connections underlying modulation of visual cortical oscillations. Subjects performed a cued attentional task during MEG recordings, requiring covert attention to left and right visual hemifields in order to categorize incoming target stimuli. We hypothesized that the medial SLF branch (SLF1) - connecting superior frontal to parietal cortex - was the structural pathway for control signals from the FEF, and that therefore individual differences in SLF1 properties would predict modulation of visual cortical oscillations. MEG analysis revealed expected modulations of both alpha and gamma oscillations. Notably, hemispheric modulation varied across subjects; in some subjects stronger modulation was observed in the left hemisphere and in others the right hemisphere. HARDI analysis revealed volumetric asymmetry in SLF1, with some subjects having greater tract volume in the left hemisphere and others in the right hemisphere. Crucially, the degree of this volumetric asymmetry was found to correlate strongly with hemispheric asymmetries in modulation of both alpha and gamma oscillations in parieto-occipital cortex. Surprisingly, SLF1 asymmetry was also found to correlate with alpha and gamma modulation asymmetries in superior frontal cortex in the vicinity of FEF. These findings provide evidence for a cortico-cortical attentional network in which the SLF acts as a conduit for top-down control signals from FEF to instantiate modulation of alpha and gamma oscillations in parieto-occipital cortex. This provides experimental support for the notion that modulation of visual cortical oscillations is the mechanism by which the DAN asserts goal-directed attention. The inability to properly modulate posterior alpha seen in persons with ADHD may have an anatomical basis detectable with diffusion MRI.

Disclosures: **T.R. Marshall:** None. **T.O. Bergmann:** None. **O. Jensen:** None.

Nanosymposium

205. Working Memory

Location: 147A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 205.11

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH095984

Title: What are the neurophysiological bases of attended and unattended items in short-term memory? An fMRI/EEG/TMS study

Authors: *N. ROSE[1], J. L. LAROCQUE[2], A. C. RIGGALL[3], O. GOSSERIES[1], B. R. POSTLE[4];
[1]Dept. of Psychiatry, [2]Med. Sci. Training Program, [3]Dept. of Psychology, [4]Dept. of Psychology and Psychiatry, Univ. of Wisconsin-Madison, Madison, WI

Abstract: Multivariate pattern analysis (MVPA) has failed to find evidence for an active neural trace of stimuli that are in short-term memory (STM) but outside focal attention (e.g., LaRocque et al., 2013). How are items maintained "in STM", if not by an active trace? The Synaptic Theory posits that items in STM but outside focal attention are retained by a passive mechanism, such as a latent network of potentiated synapses. According to this account, a latent pattern of these synaptic weights could effectively be read out by passing a nonspecific sweep of activity through the network. To evaluate this possibility, we employed a two-step experimental procedure. In an initial fMRI session, we scanned participants while they performed a single-item delayed-recognition task requiring the maintenance of a word, a face, or a direction of moving dots, and applied MVPA to identify category-selective regions for subsequent targeting with TMS. In a subsequent session, we simultaneously recorded EEG and applied single-pulse TMS while participants performed a two-item delayed recognition task with two retro-cues and two memory probes. Following initial presentation of two sample items (e.g., a word and a face), a retro-cue indicated which was to be maintained in focal attention for the first probe; the other item had to be maintained in STM, because there remained a 50% likelihood that it would be cued as the target of the second probe. By stimulating a category-selective region with a single-pulse of TMS during the delay, we could assess the physiological state of the neural representation of information when it was in one of three states: in focal attention (an attended memory item; AMI); in STM but not in focal attention (an unattended memory item; UMI); or not in STM (i.e., not present on that trial). We analyzed the delay-period EEG data with MVPA to decode the presence of category-specific patterns of neural oscillations. Decoding accuracy was significantly better than chance for AMIs, but not for UMIs. Critically, however, there was a brief recovery of MVPA decoding of the UMI when the single pulse of TMS was applied. The recovery of decoding accuracy seemed to be driven by oscillations in the beta band, whereas oscillations in the theta and alpha bands contributed to decoding items in focal attention. The recovery of decoding accuracy of the UMI is consistent with the synaptic theory of STM. It suggests that when storage is dissociated from attention, that storage in STM may be accomplished via passive retention mechanisms, such as short-term potentiation of synaptic weights in the network representing that item.

Disclosures: **N. Rose:** None. **J.L. LaRocque:** None. **A.C. Riggall:** None. **O. Gosseries:** None. **B.R. Postle:** None.

Nanosymposium

205. Working Memory

Location: 147A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 205.12

Topic: F.01. Human Cognition and Behavior

Support: NIMH R01-MH087214

ONR N00014-12-1-0972

Title: Pre-trial neural indicators of lapses in working memory performance

Authors: *K. ADAM, E. K. VOGEL;
Psychology, Univ. of Oregon, Eugene, OR

Abstract: While change detection measures of capacity require estimating performance across multiple trials, whole-report measures allow for trial-by-trial characterization of performance states. Here, we utilized the temporal precision of whole-report to look for neural predictors of lapses in performance. Participants completed 540 trials of a 6-item whole-report memory task while electroencephalography activity was recorded. During the task, subjects were briefly shown (250 ms) an array of six brightly-colored items, remembered the stimuli for a brief period (1,300 ms) and then reported the color of all six items using the computer mouse. Analysis of oscillatory power as a function of performance level revealed modulation in theta power (4-7 Hz) that was localized to frontal electrodes (F3, Fz, F4); increased frontal theta power was associated with increased performance (more items correct) on the whole-report task. Frontal theta has been previously shown to correspond with attentional state. Here, we show evidence that trial-by-trial fluctuations in pre-trial theta predict subsequent performance on a working memory task. Similarly, analysis of event-related potentials revealed a sustained increase in a negative slow-wave during the pre-trial period for successful trials relative to unsuccessful trials. These findings are consistent with previous studies of the contingent negative variation (CNV) component, where enhanced negativity of an inter-stimulus slow wave is associated with speeded reaction times to the response target. Together, these neural indicators may serve as powerful tools for predicting, and perhaps preventing, brief lapses of attention that lead to poor working memory performance.

Disclosures: K. Adam: None. **E.K. Vogel:** None.

Nanosymposium

205. Working Memory

Location: 147A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 205.13

Topic: F.01. Human Cognition and Behavior

Support: Wellcome Trust Grant CQRTDY0 to N Myers

Title: Top-down control over the contents of visual working memory is reflected in neural oscillations measured by magnetoencephalography

Authors: *N. MYERS[1,2], M. G. STOKES[2], T. WILDEGGER[1], A. C. NOBRE[1,2];
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Abstract: When we retain information in working memory (WM), we can update new items to the currently held set. This ability comes at the expense of what is already in working memory - we tend to forget previously encoded memories. To control working memory updating, therefore, new information should only be encoded if it is likely to be behaviorally relevant. We explored strategic updating in working memory with magnetoencephalographic (MEG) and functional magnetic resonance imaging (fMRI) data from the same 20 healthy young volunteers as they performed a cued visual working memory precision task. On each trial, subjects encoded orientation information from a first display into working memory. After a delay, a central cue indicated whether the previous item (protect condition) or the currently presented item (update condition) would be probed. After a further memory delay, observers estimated the remembered angle with a continuous response. In the fMRI experiment, we found that both protecting and updating activated a dorsolateral fronto-parietal network commonly linked to top-down control. In addition, protect cues activated cingulo-opercular areas, possibly reflecting the retrieval of previously encoded information. Induced oscillatory responses in the MEG signal showed that both protecting and updating led to transient desynchronization in the alpha- (8-14 Hz) and beta-bands (16-32 Hz), primarily over parietal sensors. Protecting the current contents of WM led to additional desynchronization at these frequencies at frontal-central sensors, paralleling the responses in anterior cingulate and insula seen in fMRI. These results are in line with the proposal that fronto-parietal control networks use synchronization to retrieve relevant sensory information for the optimization of behavior.

Disclosures: **N. Myers:** None. **M.G. Stokes:** None. **T. Wildegger:** None. **A.C. Nobre:** None.

Nanosymposium

205. Working Memory

Location: 147A

Time: Sunday, November 16, 2014, 1:00 PM - 4:30 PM

Presentation Number: 205.14

Topic: F.01. Human Cognition and Behavior

Support: Natural Sciences and Engineering Research Council of Canada

Canadian Institutes of Health Research

EJLB Foundation

Title: Partially-segregated neural substrates of working memory and attention to visual features in lateral prefrontal cortex neurons

Authors: *D. MENDOZA-HALLIDAY[1,2], J. C. MARTINEZ-TRUJILLO[1];
[1]McGill Univ., Montreal, QC, Canada; [2]MIT, Cambridge, MA

Abstract: The primate lateral prefrontal cortex (LPFC) is thought to play an important role in the maintenance of working memory representations of non-spatial visual features (i.e., feature working memory or FWM) as well as in the allocation of attention to features of stimuli readily available to the eyes (i.e., feature-based attention or FBA). One issue that remains unclear is whether these two functions have the same or different neural substrates within the LPFC. Here we investigated this issue in macaque monkeys by recording the responses of the same LPFC neurons during two different tasks: a FBA task, which required attending to the motion direction of a visual stimulus that remained always visible on a computer display; and a FWM task, which required maintaining a memory representation of the stimulus direction after the stimulus became visually unavailable. We found that nearly half of the recorded neurons showed selectivity for the stimulus direction during either task. Of these neurons, 33% were selective during FBA, 38% during FWM, and the remaining 29% during both FBA and FWM. Neuronal activity was predictive of trial outcome (correct vs. incorrect responses) in both tasks, but was significantly more predictive in the FWM than in the FBA task. Finally, we found that neurons selective during both tasks were clustered around a similar sub-region within the LPFC. These results support the involvement of LPFC in both working memory and attention to visual features, and show that these two processes are carried out by distinct, yet partially-overlapping, populations of neurons.

Figure 1. Behavioral tasks.

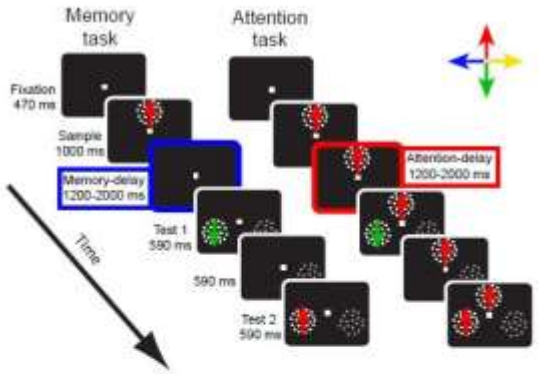
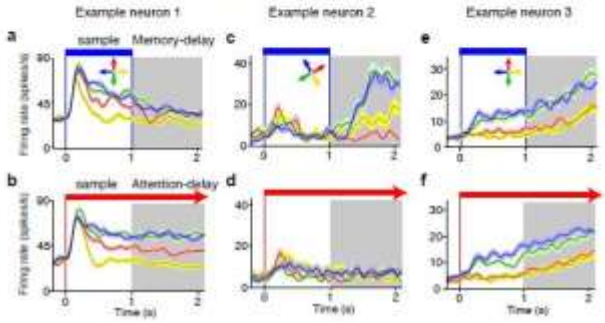


Figure 2. Activity of example neurons.



Disclosures: D. Mendoza-Halliday: None. **J.C. Martinez-Trujillo:** None.

Nanosymposium

206. The Neural Basis of Reward-Based Decisions

Location: 140A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 206.01

Topic: F.03. Motivation and Emotion

Support: ERC Biomotiv

Title: Do VMPFC neurons really care about outcome value?

Authors: *S. BOURET, A. SAN GALLI, R. ABITBOL, M. PESSIGLIONE;
Motivation Brain and Behavior, Inst. du Cerveau et de la Moelle Epiniere, Paris, France

Abstract: We all regulate our behavior as a function of the expected value of our actions. The computation of outcome value is thought to rely heavily upon the ventro-medial prefrontal cortex

(VMPFC), but the exact nature of the underlying neuronal processes remain unclear. We recorded the activity of VMPFC neurons in monkeys performing a grip force task where the amount of reward and the amount of effort required for reward obtainment were manipulated systematically. The activity of VMPFC neurons showed very little modulation by visual information about reward and effort, which was nonetheless used by monkeys to adjust their behavior. VMPFC activity was not strongly related to behavioral activation (force produced) or autonomic response (pupil dilation) either. However, there was a strong modulation by internal factors of outcome value, such as satiety level/fatigue. In addition, the global firing of VMPFC neurons was positively associated with the propensity to perform the task: the higher the population signal, the more likely the monkeys were to engage in the task and exert the required effort. Furthermore, this positive relation between neural activity and behavioral engagement was present before presentation of visual information about reward and effort levels. Thus, our data indicate that the firing of VMPFC neurons is strongly associated with internal determinants of subjective valuation and decision making.

Disclosures: **S. Bouret:** None. **A. San Galli:** None. **R. Abitbol:** None. **M. Pessiglione:** None.

Nanosymposium

206. The Neural Basis of Reward-Based Decisions

Location: 140A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 206.02

Topic: F.03. Motivation and Emotion

Support: K99 R00 (DA027718)

NARSAD Young Investigator Reward from the Brain and Behavior Research Foundation

Sloan Foundation Fellowship

Title: Value comparison through mutual inhibition in a corticostriatal circuit

Authors: ***C. E. STRAIT**[1], B. J. SLEEZER[2], T. C. BLANCHARD[1], B. Y. HAYDEN[1];
[1]Brain and Cognitive Sci., [2]Neurosci. Grad. Program, Univ. of Rochester, Rochester, NY

Abstract: Decision-makers must often choose from among competing options based on the rewards they offer. Although reward-based choice is an important part of our basic cognitive repertoire, we know little about the processes by which our brains evaluate offers, compare their values, and select a preferred option. Converging evidence from neuroimaging and lesion studies

suggests that the ventromedial prefrontal cortex (vmPFC) and the ventral striatum (VS) both play central roles in this process. We hypothesized that neurons in these areas represent abstract values (i.e., combining across value dimensions) and compare them via mutual inhibition. In this type of process, sets of single neurons 'compete' for their preferred option by suppressing each other's activity. We recorded neuronal responses from both regions while monkeys performed a two-option gambling task with asynchronous option presentation. We observed four neuronal signatures of comparison via mutual inhibition: (1) encoding of abstract values, as opposed to separate coding of reward and probability, (2) encoding of the difference between the two offered values, (3) selectivity for chosen, as opposed to unchosen options, and (4) choice probability correlates. Relative to vmPFC, the effects we observed in VS occurred at roughly the same frequency, although they were slightly more common in VS. We did not observe any qualitative differences between vmPFC and VS response properties. These findings suggest that choice is not the exclusive domain of a specific region of the cerebral cortex, but is an emergent product of interactions between cortex and the striatum.

Disclosures: C.E. Strait: None. **B.J. Sleezer:** None. **T.C. Blanchard:** None. **B.Y. Hayden:** None.

Nanosymposium

206. The Neural Basis of Reward-Based Decisions

Location: 140A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 206.03

Topic: F.03. Motivation and Emotion

Support: Henry Wellcome Fellowship 098830/Z/12/Z

Title: Information search strategies during multi-attribute choice

Authors: *L. T. HUNT, N. MALALASEKERA, D. GRIGAT, R. J. DOLAN, S. W. KENNERLEY;
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Abstract: A central feature of many real-world decisions is that each alternative consists of several attributes. Such multi-attribute decisions may be evaluated in many different ways - ranging from attribute-based strategies (such as elimination-by-aspects) to alternative-based strategies (such as weighted adding of attributes). These strategies make opposing predictions as to how information will be acquired during decision formation, and, importantly, to how neural

circuits can implement the decision process. Here, we consider what normative principles might govern information search strategies in a multi-attribute choice task, and whether these match with empirical observations in humans and macaques. Two choice alternatives, consisting of two attributes, were presented. Subjects sequentially selected which feature of each alternative they wished to reveal; they could also terminate information sampling in order to make a choice. A dynamic programming approach could be adopted to derive the optimal strategy for calculating the value of gathering information. The dynamic programming model provides normative predictions of both *when* information sampling should be terminated, but also *which* information is most valuable to sample, if reward is to be maximised. We probed information gathering behaviour of both human and macaque subjects on analogous versions of the decision task. Human data was collected from a large subject pool (>8,000 participants) via a smartphone app, and compared to lab data from a smaller subject pool (21 participants, collected during magnetoencephalography). Choice data from two macaque monkeys was collected during electrophysiological investigation from prefrontal cortex. Some key aspects of behaviour matched well with predictions from the normative model. For example, subjects would terminate information sampling early if informative cues had been received that made one alternative much more likely to be rewarding. However, there were also intriguing and unambiguous violations of the normative model. For example, human subjects were found to be particularly biased toward reducing uncertainty about the value of the currently preferred alternative, even if other information searches would prove more valuable. Macaque subjects showed a similar bias, in that they would often be unwilling to terminate a decision when confronted with one very poor alternative, without first ascertaining information about the other alternative. These results imply sub-optimal information seeking behaviors that do not maximise expected reward but instead reduce uncertainty about the alternative that is to be approached.

Disclosures: L.T. Hunt: None. **N. Malalasekera:** None. **D. Grigat:** None. **R.J. Dolan:** None. **S.W. Kennerley:** None.

Nanosymposium

206. The Neural Basis of Reward-Based Decisions

Location: 140A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 206.04

Topic: F.03. Motivation and Emotion

Title: Adaptive value coding and the optimality of choice

Authors: *K. LOUIE, M. GRUBB, P. W. GLIMCHER;
Ctr. Neural Sci., New York Univ., NEW YORK, NY

Abstract: Organisms in dynamic environments face constantly changing conditions. Given finite constraints in neural capacity, efficient coding theories require that neural systems adapt to the local statistics of the environment, a well-known phenomenon in sensory circuits. Adaptation also occurs in reward-processing and decision-related brain areas, but the computational benefits and behavioral consequences of adaptive value coding are unknown. Here, we show that adaptive value coding can be implemented via divisive normalization, a canonical neural computation widely observed in sensory processing and recently shown to operate in cortical action selection circuits. To examine the relationship between adaptation and normalization, we develop a simple history-dependent firing rate model of value coding and option selection. This adaptive normalization model captures previously-reported range adaptation in orbitofrontal neuron firing rates. Furthermore, this model predicts adaptive changes in choice behavior specific for the predominant rewards in a given context. These findings suggest that a canonical gain control algorithm, divisive normalization, may mediate adaptive value coding in decision circuits and predict specific benefits of such a computation for adaptive choice behavior.

Disclosures: K. Louie: None. **M. Grubb:** None. **P.W. Glimcher:** None.

Nanosymposium

206. The Neural Basis of Reward-Based Decisions

Location: 140A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 206.05

Topic: F.03. Motivation and Emotion

Support: R00 (DA027718)

NSF CAREER aware to BYH

NARSAD Young Investigator award to BYH

Title: Coding formats during anticipatory delay reveal regulatory role of anterior cingulate cortex

Authors: *T. BLANCHARD[1], B. HAYDEN[2];
[1]Brain and Cognitive Sci., [2]Univ. of Rochester, Rochester, NY

Abstract: Competing accounts of anterior cingulate cortex (ACC) function emphasize its role in value signaling and executive control respectively. Here we use apopulation-level pattern analysis approach to examine single-unit responses in a delayed reward task. We find that, while neural activity correlated with the reward expected throughout the delay, the effect that reward has on firing rates changes continuously over time, suggesting that activity is driven more by time-varying task demands than by reward itself. Interestingly, for both safe and uncertain outcomes, responses immediately before the reward, but not earlier, simulate the post-reward response, suggesting that anticipatory ramp-up of firing serves to predict control arising from anticipated outcomes. We also find that neural activity in anticipation of uncertain outcomes does not signal subjective or expected value. These results suggest that dACC activity is only incidentally correlated with reward amounts and is more closely associated with executive control. These results suggest that dACC activity is only incidentally correlated with reward amounts and is instead more closely associated with deployment of executive control.

Disclosures: **T. Blanchard:** None. **B. Hayden:** None.

Nanosymposium

206. The Neural Basis of Reward-Based Decisions

Location: 140A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 206.06

Topic: F.03. Motivation and Emotion

Support: NIMH Caltech Conte Center

Title: Characterizing value-related responses in monkey orbital and medial prefrontal cortices using fMRI

Authors: *S. COLLETTE, T. LARSEN, E. LAWLER, N. SCHWEERS, J. P. O'DOHERTY, D. Y. TSAO;
Caltech, Pasadena, CA

Abstract: Considerable progress has been made in identifying neural correlates of value and value-based decision making in both monkey and human brains. A particular focus in the literature has been on the contribution of the ventral prefrontal cortex, with findings from neurophysiological recording studies in monkeys and fMRI studies in humans supporting a role for this part of the brain in encoding the value of stimuli and/or goods around the time of decision-making. However, the monkey and human literatures often differ considerably in terms

of the precise neuroanatomical locations of the relevant neuronal populations or activation clusters implicated in this process. Monkey recordings are typically obtained from central to lateral parts of the orbitofrontal cortex (OFC), while in humans the medial OFC and adjacent medial prefrontal cortex is most often implicated in value-related processing. One major open question in the literature is whether or not apparent differences across species in the location of value-related signals within ventral prefrontal cortex represent actual functional neuroanatomical differences between the species. Alternatively, such discrepancies could be merely due to methodological reasons: a preference among researchers as to where neurophysiological data is typically recorded in monkey OFC or underlying differences between the types of signals that can be detected with fMRI compared to with neurophysiology. To address these questions it is imperative to acquire the same type of data in both monkeys and humans using similar tasks, and similar methodologies. Here, we report results from a macaque who underwent repeated fMRI scans while performing a simple binary decision-making task that is analogous to that performed in many human studies of decision-making involving fMRI. Using MION contrast, we were able to test for activation clusters correlating with the value of the chosen option during the decision phase, which is a signal often found in human fMRI studies. Preliminary analyses reveal that value-related activations are most prominently located in central and lateral parts of OFC, in strikingly similar locations to where relevant neuronal responses are typically reported in monkeys. On the other hand, little activity was found in ventromedial prefrontal cortex in this contrast. On the contrary, when testing for reward prediction errors we found activations in ventral striatum as is typically reported in human fMRI studies, as well as a region of medial OFC. This approach when combined with other techniques, has the potential to provide important insights into homologies in value-based decision-making processes across species.

Disclosures: **S. Collette:** None. **T. Larsen:** None. **E. Lawler:** None. **N. Schweers:** None. **J.P. O'Doherty:** None. **D.Y. Tsao:** None.

Nanosymposium

206. The Neural Basis of Reward-Based Decisions

Location: 140A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 206.07

Topic: F.03. Motivation and Emotion

Support: ZIA MH002928-01

Title: Neural encoding of the immediate and future value of novel choice options in amygdala, ventral striatum, and orbitofrontal cortex

Authors: *V. D. COSTA, L. C. KAKALIOS, B. B. AVERBECK;
Lab. of Neuropsychology, NIMH/NIH, Bethesda, MD

Abstract: Novelty seeking refers to the tendency of humans and other animals to explore novel and unfamiliar stimuli and environments in pursuit of potential reward. It therefore represents a specific case of the explore/exploit dilemma that underlies decisions between multiple choice options. From this perspective the utility of exploring novel options can be formally characterized as a combination of its immediate expected value (IEV) and its future expected value (FEV). FEV characterizes the value of future actions that can be made after choosing an option and receiving feedback. We used a finite state, discrete time, infinite-horizon Markov decision process (MDP) to derive IEV and FEV estimates for each of the monkeys' choices. To examine neural encoding of the immediate and future value of choice options, we simultaneously recorded single-unit neural responses in amygdala, ventral striatum, and orbitofrontal cortex in three rhesus macaques as they played a three armed bandit task. During the task, the monkeys learned to choose between three, probabilistically rewarded images. Periodically one of the three choices was replaced with a novel image the monkey had not yet associated with reward. We then determined the extent to which IEV and FEV were represented by single neurons in each brain region. Results indicated that IEV and FEV were significantly encoded in each region. At the population level IEV of the chosen option was more strongly encoded in the amygdala than orbitofrontal cortex or ventral striatum. The FEV, on the other hand, was more strongly encoded in the amygdala and ventral striatum than in orbitofrontal cortex. Finally, we also used a multinomial regression model to characterize the effects of IEV and FEV on the monkeys' selection of novel versus familiar options. Use of value regressors modified by their impact on behavioral choice led to an increase in the fraction of neurons significantly encoding each value signal. Together these results suggest that amygdala, ventral striatum, and orbitofrontal cortex contribute to a neural circuit that drives novelty based, exploratory decision making.

Disclosures: V.D. Costa: None. **L.C. Kakalios:** None. **B.B. Averbeck:** None.

Nanosymposium

206. The Neural Basis of Reward-Based Decisions

Location: 140A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 206.08

Topic: F.03. Motivation and Emotion

Support: Wellcome Trust

Title: Competing strategies for reward learning in the orbitofrontal cortex and connected brain regions

Authors: *A. O. CONSTANTINESCU[1], G. JOCHAM[2], A. M. IANNI[1,3], T. E. J. BEHRENS[1,4]; [1]Oxford Ctr. for Functional MRI of the Brain, Oxford, United Kingdom; [2]Ctr. for Behavioral Brain Sci. and Fac. of Economics, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany; [3]Natl. Inst. of Mental Health, NIH, Bethesda, MD; [4]The Wellcome Trust Ctr. for Neuroimaging, Univ. Col. London, London, United Kingdom

Abstract: Our daily behaviour is guided by our ability to learn which stimuli and actions lead to rewarding outcomes. Multiple learning systems exist in the brain and, when disrupted, they lead to unusual behaviours. Lesions in the orbitofrontal cortex (OFC) lead to worse choices and subjects paradoxically choose non-rewarding stimuli if they used to be rewarding in the past [1]. Intriguingly, amygdala lesions reverse these deficits [2] and even lead to better choices in subjects with an intact OFC [3]. The amount of influence each learning system has on behaviour remains unknown. Here, we aimed to investigate the main learning mechanisms that vary naturally in their contribution to guide human behaviour, and their neural underpinnings. Subjects (n=23) performed a novel reward-guided learning task during functional magnetic resonance imaging. Three shapes were sliding on screen for 1.5 seconds and subjects chose each of them with a different finger press. Each shape had an independent reward probability that changed over time. Subjects received a contingent reward **(CR)** 3 seconds after they chose a rewarding shape. They also received free rewards **(FR)** of a different colour, given independently of their behaviour. Crucially, subjects were precisely instructed to focus on **CR** and to ignore the **FR**. We found three independent strategies that drove behaviour. Firstly, **contingent learning** was the dominant and optimal strategy, which made precise associations between **CR** and their causal choices. Secondly, **recency learning** assigned **FR** to previous, immediate choices. Thirdly, **average history learning** assigned rewards to stimuli chosen many times in the past. We identified a signature of **contingent learning** in the OFC and caudate nucleus ($p<0.05$, cluster corr). We also found a measure of **recency learning** in the putamen and premotor cortex ($p<0.05$, cluster corr). Interestingly, subjects with stronger OFC responses had less pronounced **recency learning** ($r=-0.44$, $p=0.017$) and **average history learning** ($r=-0.48$, $p<0.01$). Moreover, subjects with more OFC activation during amygdala deactivation, as measured by functional connectivity, had more **contingent learning** ($p<0.05$, cluster corr). We present behavioural and neurobiological evidence for three competing learning mechanisms that vary naturally in their contribution to guide human behaviour. Our results suggest that humans with more OFC activity have a more optimal behaviour because they make precise associations between stimuli and outcomes. [1] Izquierdo et al, J Neurosci, 2004 [2] Stalnaker et al, Neuron, 2007 [3] Rudebeck et al, J Neurosci, 2008

Disclosures: **A.O. Constantinescu:** None. **G. Jocham:** None. **A.M. Ianni:** None. **T.E.J. Behrens:** None.

Nanosymposium

206. The Neural Basis of Reward-Based Decisions

Location: 140A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 206.09

Topic: F.03. Motivation and Emotion

Support: Wellcome Trust New Investigator Award

Title: Neuronal correlates of value depend on information gathering and comparison strategies

Authors: ***N. MALALASEKERA**[1], L. T. HUNT[1,2], B. MIRANDA[1], T. E. BEHRENS[2], S. W. KENNERLEY[1];
[1]Sobell Dept. of Motor Neurosci. and Movement Disorders, London, United Kingdom;
[2]Wellcome Trust Ctr. for Neuroimaging, Inst. of Neurol., London, United Kingdom

Abstract: Animal and human lesion studies describe a double dissociation between anterior cingulate cortex (ACC) and orbitofrontal cortex (OFC) damage; ACC lesions cause deficits in action based decision making, whereas OFC lesions profoundly disrupt stimulus based decision making. This suggests neuronal populations within different prefrontal subregions may represent relevant decision variables in different value reference frames. It is unknown, however, how activity in these regions evolves during ecological situations in which choice information is sequentially gathered about distinct stimuli and actions to guide a final decision. To study value based decision-making during information gathering, monkeys were first taught a set of picture value associations for two distinct attributes (reward probability and magnitude). Subjects were then presented with decisions between two options, each comprising two learnt pictures (one from each attribute). They made choices between left and right options. Importantly, subjects were free to shift their attention (saccade) to the different pictures to gather information about attributes/options, before indicating their choice by moving a joystick to the left/right. Eye movements provided a proxy for the information gathering strategies influencing decision-making. Each cue therefore possessed three distinct attributes: its value, whether it was associated with a left/right action, and whether it was a probability or magnitude stimulus. Single neurons were recorded from ACC, OFC, lateral PFC (LPFC) and ventromedial PFC (vmPFC) while subjects performed the task. When the first picture cue was attended, a significant proportion of neurons throughout all four brain areas encoded its value. However, a population

of neurons specific to ACC and LPFC differentially encoded the value of the cue when presented on the left compared to the right, implying an action frame of reference for these neurons. In contrast, a population of OFC neurons differentially encoded the value of the cue depending on whether it was a probability or magnitude stimulus, replicating the lesion-based double dissociation between these two regions. Responses to subsequently attended cues in OFC and ACC maintained this double dissociation of value reference frames throughout the trial, but value signals evolved from encoding the value of what was currently attended to what would be eventually chosen. Furthermore, neurons in OFC differentially responded based on subsequent information gathering behaviour. This suggests that value computation and comparison may be intimately linked with information gathering strategy.

Disclosures: **N. Malalasekera:** None. **L.T. Hunt:** None. **B. Miranda:** None. **T.E. Behrens:** None. **S.W. Kennerley:** None.

Nanosymposium

206. The Neural Basis of Reward-Based Decisions

Location: 140A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 206.10

Topic: F.03. Motivation and Emotion

Support: Intramural Research Program of the National Institute of Mental Health

Title: The role of the macaque orbital prefrontal cortex in learning and reversing probabilistic reward associations

Authors: *P. H. RUDEBECK[1,2], E. A. MURRAY[3];
[1]Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]Section on the Neurobio. of Learning and Memory, Lab. of Neuropsychology, [3]Lab. of Neuropsychology, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: The orbitofrontal cortex (OFC) has long been associated with reward-guided behavior, but its specific role is still uncertain. In a study using excitotoxic lesions in macaques, it has recently been shown that the OFC is not necessary for flexibly altering behavior during object discrimination reversal learning (Rudebeck et al., 2013). Such a finding argues against the 'inhibitory control' hypothesis of OFC function. Because the findings are at odds with a large body of previous work, and because it is possible that OFC plays a role in establishing and altering stimulus-reward associations in other settings, we explored the role of OFC in more

demanding reversal learning tasks. Specifically, we tested macaque monkeys with excitotoxic lesions of the OFC (n = 4, Walker's areas 11,13, and 14) in two separate probabilistic task settings and compared their performance to a group of unoperated controls (n=8). In the first setting, macaques were presented with two stimuli on a touch screen monitor; one option was associated with 0.75 probability of receiving a reward and the other with a 0.25 probability. Over successive trials, macaques learned to select the stimulus associated with the higher probability of reward. Once they were consistently selecting the high-probability option, the stimulus reward contingencies were reversed. We collected data for 9 consecutive serial reversals. In the second setting, macaques were trained on a 3-choice probabilistic task. Here, the probability of receiving a reward for selecting any one of three distinct stimuli fluctuated over the course of a session. On both tasks, monkeys with excitotoxic lesions were no different to unoperated controls, being able to learn and track the probability of reward associated with different options and to select those associated with the highest probability of reward. Taken together, these data suggest that the macaque OFC is not necessary for probabilistic learning and reversal. We are currently assessing the contribution of parts of the prefrontal cortex outside the OFC to this type of reward-guided behavior.

Disclosures: P.H. Rudebeck: None. **E.A. Murray:** None.

Nanosymposium

206. The Neural Basis of Reward-Based Decisions

Location: 140A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 206.11

Topic: F.03. Motivation and Emotion

Support: Hilda and Preston Davis Foundation

NIDA Grant R01 DA19028

NINDS Grant P01 NS040813

Title: Evidence for directionality in OFC high-frequency oscillations

Authors: *E. L. RICH, J. D. WALLIS;
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Abstract: Neurons in the orbitofrontal cortex (OFC) are modulated by stimulus value. A standard view holds that OFC computes stimulus values by combining highly processed sensory

inputs with other information such as current context, internal states, and semantic and episodic memories, and the resulting signal is a subjective and temporally-specific outcome prediction used to guide behavior. Because sensory afferents enter OFC posteriorly, this framework assumes that the flow of information should be from posterior to anterior. In order to assess this hypothesis, we recorded single neurons and local field potentials (LFPs) from multiple sites spanning 11mm of the anterior-posterior extent of the macaque OFC. Subjects were trained in a reward preference task, in which they chose between picture stimuli that predicted rewards of different types (primary versus secondary) and different amounts. When presented with a choice between two stimuli, subjects consistently chose pictures that predicted the larger reward regardless of reward type. During recording, we assessed neural responses to forced-choice trials, in which the subject was shown only one reward-predicting picture, and received the corresponding outcome. Among single neurons, 33% encoded the predicted reward value, regardless of reward type, and 14% encoded the value of only one type of reward. We also found that LFP oscillations in the gamma range (60-100 Hz) encoded the predicted reward value, but did not differentiate reward type. Among value coding neurons, 51% increased firing rates when larger rewards were expected, while 49% increased firing rates for smaller rewards. In contrast, the amplitude of gamma oscillations always increased when the subject expected larger rewards. In addition, this gamma response appeared earliest at the most anterior OFC sites, and the response latency progressively increased as one moves posteriorly within OFC. Therefore value information is represented at the level of the LFP, and appears to propagate in an anterior to posterior direction, opposite of the directionality predicted by the standard view of OFC processing. Instead, our results may be evidence of a top-down flow of value information within OFC.

Disclosures: E.L. Rich: None. **J.D. Wallis:** None.

Nanosymposium

206. The Neural Basis of Reward-Based Decisions

Location: 140A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 206.12

Topic: F.03. Motivation and Emotion

Support: NIH RO1 EY018620

NIH P50 MH45156

NIH P30 EY08098

NIH P41RR03631

Title: Neuronal encoding of rewards and penalties in the primate amygdala: Strong and consistent reward signals versus weak and inconsistent penalty signals

Authors: *M. L. LEATHERS[1,2], C. R. OLSON[1,2];
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Abstract: Neurons in the amygdala of the rhesus monkey respond with differential strength to cues predicting rewards and penalties. Some neurons fire more in response to cues predicting rewards while others fire more in response to cues predicting penalties. It has been suggested that reward-preferring neurons encode appetitive value whereas penalty-preferring neurons encode aversive value. However this idea has not been put to the test through the use of cues associated with rewards of different size and penalties of different size. Accordingly, we trained a monkey to perform a task requiring it to choose between cues associated with four possible outcomes. These were a small or large reward (one or three drops of water) and a small or large penalty (400 or 3200 ms of time out). On each trial, two familiar visual cues consistently associated with different outcomes were displayed to the right and left of fixation. The monkey chose an outcome by making a saccade to the associated cue. The monkey behaved rationally, choosing large reward over small reward over small penalty over large penalty. Upon monitoring the activity of amygdala neurons during choice-task performance, we found that they fired during the period following the onset of the cues at a level determined by the nature of the two associated outcomes. The impact of the contralateral cue was strongest and developed earliest. Accordingly, we focused on the influence of this cue. We posed two questions. 1) Did the mean firing rate of the neuronal population encode reward size or penalty size? We found that the mean firing rate was markedly and significantly stronger (by 4.8 sp/s) for large than for small reward but was only marginally and insignificantly stronger (by 0.19 sp/s) for large than for small penalty. In an analysis based on treating the neurons as if they had been recorded simultaneously, classifiers based on mean population firing rate accurately categorized 99% of pseudo-trials as involving a large or small reward but only 55% of pseudo-trials as involving a large or small penalty. 2) Could reward size or penalty size be extracted from population activity by a decoding approach not requiring that signals carried by different neurons be of the same sign? A decoder for reward size achieved 92% accuracy whereas a decoder for penalty size achieved only 65% accuracy. We conclude that the encoding of reward size was strong and of consistent sign whereas the encoding of penalty size was weak and of inconsistent sign. These observations are difficult to reconcile with a simple scheme in which two populations of neurons encode, in an equivalent manner, reward size and penalty size.

Disclosures: M.L. Leathers: None. **C.R. Olson:** None.

Nanosymposium

206. The Neural Basis of Reward-Based Decisions

Location: 140A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 206.13

Topic: F.03. Motivation and Emotion

Title: Deletion of α5 nicotine receptor subunits abolishes nicotinic aversive motivational effects in a manner that matches dopamine receptor antagonism

Authors: *T. E. GRIEDER[1], H. VARGAS-PEREZ[2], M. CHWALEK[2], G. MAAL-BARED[3], D. VAN DER KOOY[4];
[1]Inst. Med. Sci., [2]Dept. of Mol. Genet., [3]Inst. of Med. Sci., [4]Univ. Toronto, Toronto, ON, Canada

Abstract: Drug use, specifically nicotine abuse, is a worldwide epidemic that claims millions of lives each year. Genetic deletion of the α5 nicotinic acetylcholine receptor (nAChR) subunit has been associated with increased intake of nicotine and somatic withdrawal, however, it remains unclear whether acute nicotine is less aversive or more rewarding, and whether mice lacking the α5 nAChR subunit can experience motivational withdrawal from chronic nicotine. Here we used the place conditioning and conditioned taste avoidance paradigms to examine the effect of α5 nAChR subunit knockout on nicotine motivation in nondependent and nicotine-dependent and -withdrawn mice, and compared these nicotinic motivational effects with those elicited after dopamine receptor antagonism. We showed that groups of nondependent mice that were pretreated with the dopamine receptor antagonist α-flupenthixol or that had deletion of α5 nAChR subunits (α5 -/-) found low, normally non-motivational doses of nicotine rewarding, and did not show an aversive motivational response or conditioned taste avoidance to higher aversive doses of nicotine. Furthermore, separate groups of nicotine-dependent α-flupenthixol pretreated and α5 -/- mice did not show an aversive motivational response to withdrawal from chronic nicotine. These results suggest that α5 nAChR subunits are critical for the experience of nicotine's aversive motivational effects in both a nondependent and nicotine-dependent and -withdrawn motivational state, and that genetic deletion of α5 nAChR subunits functions similarly to blocking dopaminergic signalling at dopamine receptors. Furthermore, modulation of nicotinic receptors containing α5 subunits may modify dopaminergic signalling, suggesting novel motivational treatments for smoking cessation.

Disclosures: T.E. Grieder: None. **H. Vargas-Perez:** None. **M. Chwalek:** None. **G. Maal-Bared:** None. **D. van der Kooy:** None.

Nanosymposium

207. Imaging the Healthy and the Diseased Brain

Location: 150A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 207.01

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Performance evaluation of 3d automatic neuron reconstruction approaches

Authors: *Z. ZHOU, S. SORENSEN, M. FISHER, D. SANDMAN, A. HENRY, K. JOINES, T. DESTA, J. HOHMANN, W. WAKEMAN, N. DA COSTA, L. LI, S. SUNKIN, E. LEIN, H. ZENG, M. HAWRYLYCZ, C. KOCH, H. PENG;
Allen Inst. For Brain Sci., Seattle, WA

Abstract: Reconstruction of a neuron's morphology is important for quantitative analysis of various properties of a neuron. Yet, for mammalian neurons that have complicated arborization patterns embedded in images of relatively low signal to noise ratios, *e.g.* bright field images or noise contaminated laser scanning images, it remains very time consuming to produce high quality reconstructions of neuron morphology. This has been particularly challenging in the context of large scale neuroscience initiatives such as the Allen Institute's mouse and human cell types projects. We bench-tested several of the most frequently used automated neuron reconstruction algorithms, including deformable models, graph models, and shape-fitting models using a number of neuronal images. We are especially interested in testing using the images generated from the Allen Institute's initiatives that are representatives of some really demanding use cases. Analysis of these neurons' morphologies allows us to better categorize cell types and which different salient features of neurons would be captured by these independently designed reconstruction algorithms. To make the comparison as fair as possible, we ported these algorithms and related software into the publicly available and Open Source Vaa3D software platform (http://vaa3d.org) as plugins, so that they can be tested using the same input to generate the output reconstructions in the same format. We will make the bench-test results available to the public, along with a comprehensive analysis of the results.

Disclosures: Z. Zhou: None. **S. Sorensen:** None. **M. Fisher:** None. **D. Sandman:** None. **A. Henry:** None. **K. Joines:** None. **T. Desta:** None. **J. Hohmann:** None. **W. Wakeman:** None. **N. da Costa:** None. **L. Li:** None. **S. Sunkin:** None. **E. Lein:** None. **H. Zeng:** None. **M. Hawrylycz:** None. **C. Koch:** None. **H. Peng:** None.

Nanosymposium

207. Imaging the Healthy and the Diseased Brain

Location: 150A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 207.02

Topic: G.03. Staining, Tracing, and Imaging Techniques

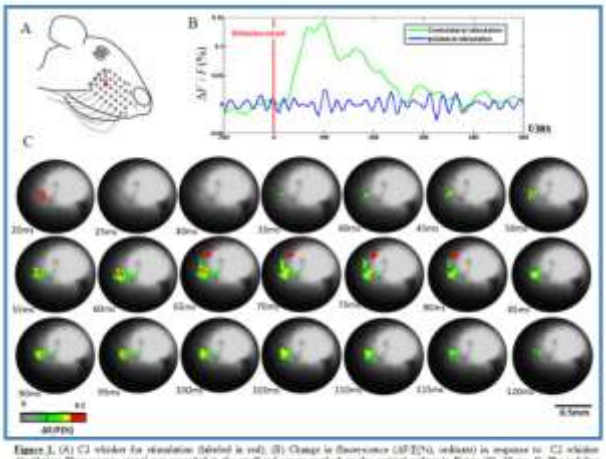
Support: NSF Career Award (Y. C.)

NIH R01NS 039050-13 (R.S.E.)

Title: *In vivo* voltage-sensitive dye optical imaging of the subcortical brain structures

Authors: *Q. TANG[1], V. TSYTSAREV[2], C.-P. LIANG[1], R. S. ERZURUMLU[2], Y. CHEN[1]; [1]Univ. of Maryland-College Park, College Park, MD; [2]Dept. of Anat. and Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Functional brain optical imaging has grown intensively within last few decades.Localization and real-time monitoring of the neural activity evoked by peripheral stimulation are important steps in understanding the functional characteristics of neuronal circuits in the brain.Although in vivo optical imaging has been pivotal in enabling studies of neural activity in the brain, conventional imaging system has generally been limited to the surface of the cerebral cortex.The rodent vibrissae system is an excellent model to investigate the development, organization,function and plasticity of mammalian sensory pathways.It's anatomical organization along the trigeminal afferent pathway conserves the functional representation of the facial whiskers:the barrelettes in the brainstem,the barreloids in the thalamus and the barrels in the contralateral primary somatosensory cortex. Voltage-sensitive dye imaging (VSDi) offers an opportunity to study the activity of neuronal ensembles in vivo with relatively high spatial (up to 20μm) and temporal resolution (up to few milliseconds) which is comparable with electrophysiology. Currently used fast CCD camera-based VSDi can only provide information from the cortical surface. Gradient refractive index (GRIN) lens that are 350-2,000μm in diameter and provide micron-scale resolution have been used in deep brain imaging with minimal injury. In our research, we combined VSDi with GRIN lens to study neural functions in mice vibrissae system. Neural activities evoked in the thalamic barreloids by single whisker stimulation were visualized in vivo. For all examined animals VSDi signals elicited in response to the whisker stimulation were observed in a cintralateral thalamus anatomically corresponding to the barreloids.Our in vivo results, for the first time, show that the combination of VSDi and GRIN rod lens allow simultaneous imaging of activity-dependent changes at subcortical and cortical levels of sensory pathways in the mammalian brain.



Disclosures: **Q. Tang:** None. **V. Tsytsarev:** None. **C. Liang:** None. **R.S. Erzurumlu:** None. **Y. Chen:** None.

Nanosymposium

207. Imaging the Healthy and the Diseased Brain

Location: 150A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 207.03

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Zolpidem reduces neuronal activity in hippocampus of freely behaving mice: A large-scale calcium imaging study using miniaturized fluorescence microscope

Authors: ***T. BERDYYEVA**[1], S. OTTE[2], L. ALUISIO[1], L. D. BURNS[2], Y. ZIV[3], C. DUGOVIC[1], K. K. GHOSH[2], M. J. SCHNITZER[4], T. LOVENBERG[1], P. BONAVENTURE[1]; [1]Neurosci., Janssen LLC (Johnson & Johnson PRD), San Diego, CA; [2]Inscopix, Palo Alto, CA; [3]Dept. of Neurobio., Weizmann Inst. of Sci., Rehovot, Israel; [4]Dept. of Applied Physics, Stanford Univ., Palo Alto, CA

Abstract: The miniaturized fluorescence microscope permits calcium-imaging studies of large ensembles of individual neurons in freely behaving mice. Here we introduce the use of this technology towards drug discovery by tracking somatic calcium dynamics in hundreds of CA1 hippocampal neurons of pharmacologically manipulated behaving mice. Concurrently with imaging we monitored the animals' locomotor activity and body temperature, while also

performing electroencephalography (EEG) and electromyography (EMG) recordings. This combined approach allowed us to control for physiological and behavioral changes often associated with drug action and thereby to reduce the ambiguity in the interpretation of observed drug effects. We used an adeno-associated viral vector to express the genetically encoded calcium sensor GCaMP3 in CA1 pyramidal cells under control of the CaMKII promoter and a miniaturized microscope to observe pyramidal cell dynamics. We visualized these dynamics with and without a systemic administration of Zolpidem, a GABAA agonist that is presently the most commonly prescribed sleep aid medication in the United States. Although there have been growing concerns about potential adverse effects of Zolpidem on memory and cognition, it has been unknown whether Zolpidem alters neuronal activity in hippocampus, a brain area critical for cognition and memory. We found that Zolpidem, when delivered at a dose previously shown to mimic human sleep-inducing effects, strongly suppressed CA1 neural activity. The rate of calcium transients after Zolpidem administration was significantly lower as compared to vehicle only. To factor out possible confounding effects of locomotor or physiological state changes following Zolpidem dosage, we compared neural activity across comparable epochs matched by locomotor and physiological assessments. This analysis revealed significantly depressive effects of Zolpidem regardless of the animal's state. Individual hippocampal CA1 pyramidal cells differed in their responses to Zolpidem. A majority (65%) significantly declined in activity, but a small subset (3%) showed a significant increase even after statistical correction for multiple comparisons. Therapeutic drugs are often characterized by their mechanism of action at the molecular level, omitting drug effects on neural circuit activity in behaving animals. Here we demonstrate a new approach to elucidating drug actions on large-scale neural activity in behaving animals. By helping to link molecular mechanisms, neural circuit dynamics and animal behavior, this approach has the potential to contribute substantially to the development of new therapeutics.

Disclosures: **T. Berdyyeva:** A. Employment/Salary (full or part-time):; Janssen LLC. **S. Otte:** A. Employment/Salary (full or part-time):; Inscopix. **L. Aluisio:** A. Employment/Salary (full or part-time):; Janssen LLC. **L.D. Burns:** F. Consulting Fees (e.g., advisory boards); Inscopix. **Y. Ziv:** F. Consulting Fees (e.g., advisory boards); Inscopix. **C. Dugovic:** A. Employment/Salary (full or part-time):; Janssen LLC. **K.K. Ghosh:** A. Employment/Salary (full or part-time):; Inscopix. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inscopix. **M.J. Schnitzer:** F. Consulting Fees (e.g., advisory boards); Inscopix. **T. Lovenberg:** A. Employment/Salary (full or part-time):; Janssen LLC. **P. Bonaventure:** A. Employment/Salary (full or part-time):; Janssen LLC.

Nanosymposium

207. Imaging the Healthy and the Diseased Brain

Location: 150A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 207.04

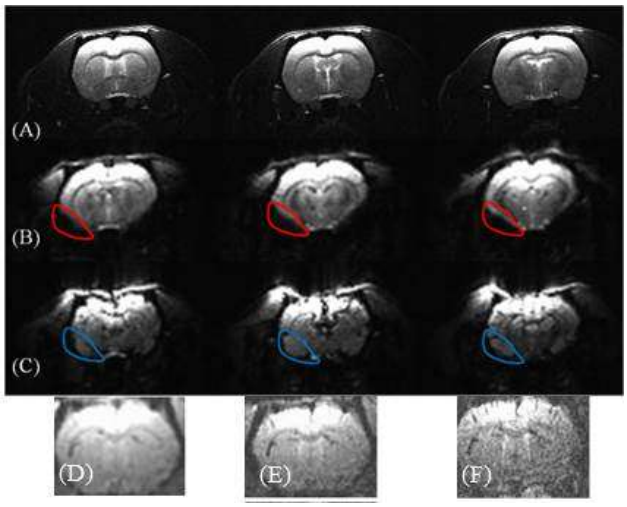
Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH Grant R01EB0000215

Title: Eliminating EPI signal drop out in insular and amygdala area in rat functional MR study- a step toward true whole brain functional imaging

Authors: *R. LI[1], X. LIU[2], P. BISHOP[1], J. SIDABRAS[1], H. S. MATLOUB[3], J. S. HYDE[1]; [1]Biophysics, [2]Dermatol., [3]Plastic Surgery, Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Self-designed 10mm diameter surface coil with low noise amplifier was used in 9.4 Tesla Bruker scanner. A tympanostomy tube was placed inside ears under microscope. Once the tympanic membrane is penetrated, the ear is filled with Fomblin Y and ear canal is sealed with cotton. Full k-space gradient echo EPI sequence was used and 10 contiguous 1 mm scans were acquired. To test the signal intensity boost, half k-space EPI sequence with 20 overscans lines was used and 20 continuous slices with slice thickness of 0.2mm was acquired. With both our self-designed 10mm coil and Bruker 20 mm coil, great deep brain coverage can be achieved (Fig 1A) with RARE sequence for anatomy. With the Bruker 20 mm coil, significant EPI signal drop out can be seen in the deep brain area especially in the insular regions. No EPI signal can be acquired from this area (Fig. 1B, red). With new method, we are able to acquire EPI signal from this area (Fig. 2C, blue) and get similar image comparing to the RARE anatomical scans. Figure 2 shows the direction comparison of the same brain slice at 300 cubic microns (D), 200 cubic microns(E) and 150*150*200 microns(F) from a single animal. Benefit from the new method, we are able to acquire high quality EPI image from the deep brain under high resolution. EPI signal drop out around insular area of rat brain has been a significant problem in rat functional MRI study. This problem is a major obstacle toward whole brain imaging study and rat connectom study. With our new technical development, we are able to acquire EPI signal from this area. Limited by the paragraph, we did not include an fcMRI analysis based on insular cortex of rat. We also tackled the SNR issue with this new setup. Depending on the imaging resolution, this new coil design yields two-to-five folds of increase in SNR comparing to the Bruker surface coil. As the result, our fMRI and fcMRI resolution can reach cortical column level from all three dimensions.



Disclosures: **R. Li:** None. **X. Liu:** None. **P. Bishop:** None. **J. Sidabras:** None. **H.S. Matloub:** None. **J.S. Hyde:** None.

Nanosymposium

207. Imaging the Healthy and the Diseased Brain

Location: 150A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 207.05

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH UL1 RR024992

ISMRM 2012 Seed grant

ICTS-WUSTL

American Cancer Society (ACS)

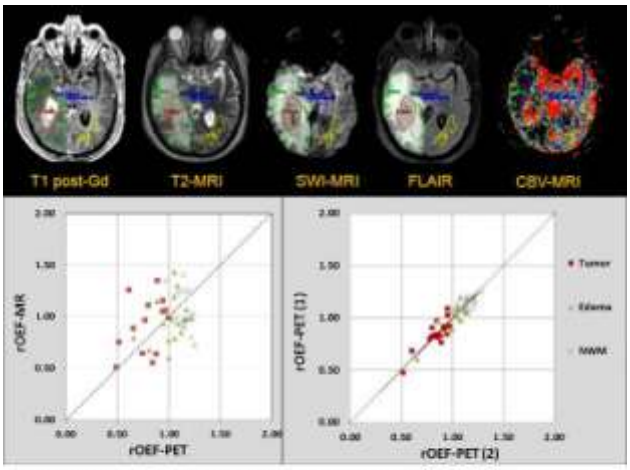
Siteman Cancer Center (58-010-52 IRG)

Title: Cerebral Oxygen Extraction Fraction (OEF) measurements obtained by MR and PET in patients with brain tumors

Authors: *P. MASSOUMZADEH[1], S. NAJMI[2], H. ANN[3], R. DHANASHREE[2], J. MCCONATHY[2], A. G. VLASSENKO[2], Y. SU[2], D. MARCUS[2], S. J. FOUKE[4], K. RICH[2], T. BENZINGER[2];

[1]Washington Univ. In St. Louis, Sch. of Medi, MO; [2]Washington Univ. In St. Louis, Sch. of Medi, St. Louis, MO; [3]Univ. of North Carolina, Chapel Hill, NC; [4]Swedish Neurosci. Specialists Ivy Brain Tumor Ctr., Seattle, WA

Abstract: Objective: To quantify and compare neuroimaging based (PET and MR) measurements of cerebral oxygen extraction fraction (OEF) in brain tumors, surrounding edema, and normal brain tissue. Methods: 30 participants (20 with brain tumors) were recruited. MRI protocol included standard clinical sequences and oxygen sensitive MR scans; a two-dimensional multi-echo gradient spin echo sequence. Concurrent with the MR acquisition, subjects with brain tumors underwent PET scanning, which included 2 sets of 3 scans with serial inhalation of air with 40-75 mCi radiolabeled carbon monoxide (C15O), 40-75 mCi radiolabeled oxygen (15O2), and injection of 25-50 mCi radiolabeled water (H215O). MR and PET data were post-processed off line and registered to the anatomic T1 pre-and post-contrast images. Regions of interest were drawn based upon contrast-enhancing tumor areas, none-enhancing T2-hyper intensity (edema), contra-lateral normal white matter (NWM), and normal gray matter (NGM). Ratios of OEF (rOEF) were obtained for lesions compared to NGM. Pearson correlations coefficients and p values were calculated. Results: There is very good correlation between two rOEF-PET measurements for enhancing tumor ($R=0.82$ slope=1.01, $p=0.00033$), none-enhancing T2-hyperinternsity ($R=0.88$ slope=0.99, $p<0.00001$), and NWM ($R=0.87$ slope=0.99, $p<0.00001$). rOEF-MR and rOEF-PET correlates well when subjects with SWI abnormalities (blood cloth, hemorrhage, calcification) are excluded, for enhancing tumor ($R=0.39$, slope=0.61, $p=0.17$) and over all ROIs ($R=0.3$ slope =0.35, and $P=0.04$). Conclusions: Both MR and 15O-PET can measure OEF in brain tumors and in peritumoral edema. Variable OEF measurements for tumor and edema may be implication for tumor grade and prognosis. BOLD MR fails in regions with signal loss on SWI or T2*. Both techniques have tremendous potential and may offer new insight into the underlying physiology of brain tumors and their response to therapy without requiring radiation or injected contrast.



Top row: Sample of registered images (T1 post-Gd, T2-MR, SWI-MR, FLAIR, and CBV-MR) and ROI's (enhancing tumor in red, none-enhancing T2-hyperintensity (edema) in green, NWM in yellow, and thalamus in blue) for a 57-year-old male with WHO grade IV glioblastoma. Bottom left row: Relative OEF of enhancing tumor (red square), edema (green triangle), and NWM (X marker) compared to thalamus for MR and PET. By excluding lesions with SWI abnormalities (blood cloth, hemorrhage, calcification) overall correlation. Bottom right row: Relative OEF value of tumor, edema, and NWM for two sets of PET measurements.

Disclosures: P. Massoumzadeh: None. **S. Najmi:** None. **H. Ann:** None. **R. Dhanashree:** None. **J. McConathy:** F. Consulting Fees (e.g., advisory boards); Speakers Bureau, Eli Lilly and Company Research Consultant, General Electric Company Research Consultant, Blue Earth Diagnostics Ltd Research Consultant, Siemens AG. **A.G. Vlassenko:** None. **Y. Su:** None. **D. Marcus:** None. **S.J. Fouke:** None. **K. Rich:** None. **T. Benzinger:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Research Grant: ACS, ICTS, NIH. F. Consulting Fees (e.g., advisory boards); Eli Lilly and Company.

Nanosymposium

207. Imaging the Healthy and the Diseased Brain

Location: 150A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 207.06

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Identification and characterization of a novel small molecule phosphodiesterase 10A PET-tracer, AMG580

Authors: *H. CHEN[1], E. HARRINGTON[2], D. LESTER-ZEINER[1], J. SHI[3], J. WONG[3], J. ABLE[1], C. BIORN[1], S. RUMFELT[2], R. KUNZ[2], T. NIEXY[2], S. MILLER[4], C. DAVIS[5], D.-R. HWANG[6], Z. YU[6], G. VARGAS[6], D. IMMKE[4], J. ALLEN[2], J. TREANOR[1];
[1]Neurosci., Amgen, INC., South San Francisco, CA; [2]Medicinal Chem., Amgen, Thousand Oaks, CA; [3]PKDM, Amgen, South San Francisco, CA; [4]Neurosciences, [5]PKDM, [6]Early Develop., Amgen, Thousand Oaks, CA

Abstract: Phosphodiesterase 10A (PDE10A) has been implicated in neurological disorders, such as schizophrenia and Huntington disease. PDE10A inhibitors have therapeutic potentials for these disorders. One of the key issues for CNS drug development is to address target coverage of therapeutic candidates in brain. A radiolabeled tracer for noninvasive imaging of target in the brain is a valuable tool for lead optimization in CNS drug discovery and for assisting dose selection in clinical development. We report the identification of a novel PDE10A tracer, AMG580, using a LC-MS/MS method. AMG580 is a small molecule PDE10A inhibitor with high affinity (double digit picomolar K_D) and high specificity (more than thousand fold selectivity over other PDEs). It was characterized extensively both *in vitro* and *in vivo.* It has improved retention time (slower target off-rate) on PDE10A and greater target to reference tissue ratio than our previously published tracer, AMG 7980. [^{18}F]-AMG580 was characterized by positron emission tomography (PET) studies in non-human primates. Excellent specific binding was observed in basal ganglia, and that is consistent with the known distribution of PDE10A in primate brain. Our results indicate that [^{18} F]-AMG580 is a potential imaging biomarker for mapping PDE10A distribution and ensuring target coverage by therapeutic candidate in the clinic.

Disclosures: H. Chen: None. **E. Harrington:** None. **D. Lester-Zeiner:** None. **J. Shi:** None. **J. Wong:** None. **J. Able:** None. **C. Biorn:** None. **S. Rumfelt:** None. **R. Kunz:** None. **T. Niexy:** None. **S. Miller:** None. **C. Davis:** None. **D. Hwang:** None. **Z. Yu:** None. **G. Vargas:** None. **D. Immke:** None. **J. Allen:** None. **J. Treanor:** None.

Nanosymposium

207. Imaging the Healthy and the Diseased Brain

Location: 150A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 207.07

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH Grant 4R33MH092797–03

Title: Comparison of two fluorine-18-labeled pet tracers for imaging pde10a

Authors: *H. JIN[1], J. LI[2], X. ZHANG[2], H. FLORES[3], Y. SU[3], J. S. PERLMUTTER[3], Z. TU[2], J. FAN[4];
[2]Dept. of Radiology, [3]Dept. of Neurol., [1]Washington Univ., Saint Louis, MO; [4]Div. of Advanced Med. Imaging Res., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Previously we have reported the radiosynthesis of the PDE10A PET tracer [^{18}F]TZ8110 that has faster washout kinetics and its radioactive metabolites do not penetrate the blood brain barrier. The goal of this study is to compare [^{18}F]TZ8110 with a new tracer [^{18}F]TZ19106B for quantifying PDE10A in brains of non-human primate (NHP). NHP microPET scans of [^{18}F]TZ8110 and [^{18}F]TZ19106B were performed to determine test and re-test variability across different subjects. The volume distribution and binding potential (BP) were calculated by Logan graphic linear regression and a two-tissue-compartment model with Simplified Reference Tissue Model (SRTM). Baseline PET studies showed that both of [^{18}F]TZ8110 and [^{18}F]TZ19106B had specific retention in the PDE10A-rich striatum, fast wash-out and a good contrast ratio of striatum-to-cerebellum. Striatum has the highest volume of distribution while the cerebellum has the lowest. Consistent non-displaceable BP_{nd} values were estimated for both modeling methods. For a 120-min dynamic acquisition, the binding potential (BP_{nd}) calculated with a Logan plot for striatum was 2.21 ± 0.58, (n = 5), and 4.32 ± 1.04 (n = 4) for [^{18}F]TZ8110 and [^{18}F]TZ19106B, respectively when using cerebellum as the reference region. These results showed good correlation with corresponding BP_{nd} values using a SRTM for [^{18}F]TZ8110, 2.31 ± 0.55 (n = 5) and [^{18}F]TZ19106B, 4.50 ± 0.81 (n = 4). The 2-tissue compartment Reference Tissue Model (RTM) was applied to estimate the k3 and k4 value for both tracers. The k3 value for [^{18}F]TZ8110 was 0.0434 ± 0.0087 min^{-1}, while the value for [^{18}F]TZ19106B was 0.0505 ± 0.0074. The k4 value for [^{18}F]TZ8110 was 0.0188 ± 0.0022 min^{-1}, while the value for [^{18}F]TZ19106B was 0.0109 ± 0.0022 min^{-1}. The increased k3 value with decreased k4 value contributed the higher binding potential for tracer [^{18}F]TZ19106B than [^{18}F]TZ8110. Therefore, [^{18}F]TZ19106B was chosen for further evaluation. Displacement experiments clearly revealed reversible kinetics for [^{18}F]TZ19106B. Different doses of MP-10 blockade (0.1, 0.3, 0.5, 1, 1.5 and 2 mg/kg) experiments showed clear dose-dependent manner by MP-10. The MP-10 dose required to achieve 50% of target occupancy (ED_{50}) was estimated at 0.271 ± 0.08 mg/Kg which corresponded to an affinity of low nanomolar for PDE10A *in vitro* binding assay. Overall, we demonstrate that [^{18}F]TZ19106B possesses good characteristics for *in vivo* quantification of the PDE10A in NHP brain by PET and [^{18}F]TZ19106B is able to provide a PDE10A signal in the striatum with good pharmacokinetic properties which warrants translational clinical investigations.

Disclosures: H. Jin: None. **J. Li:** None. **X. Zhang:** None. **H. Flores:** None. **Y. Su:** None. **J.S. Perlmutter:** None. **Z. Tu:** None. **J. Fan:** None.

Nanosymposium

207. Imaging the Healthy and the Diseased Brain

Location: 150A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 207.08

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: CIHR Operating Grant MOP-119456

Title: Predictive statistical modeling of beta-amyloid progression in APP transgenic mice

Authors: *B. J. BEDELL, K. DEDUCK, Y. ITURRIA-MEDINA, A. C. EVANS;
McGill Univ., Montreal, QC, Canada

Abstract: Introduction The epidemic spreading model (ESM) is a promising method for predicting the proposed prion-like spread of pathogenic misfolded proteins along anatomically-connected pathways in the brain. The model considers an Aß driving region (epicenter) and predicts that inter-regional deposition patterns are dependent upon a region's connectivity to this epicenter. Unlike previous models, ESM incorporates an explicit Aß clearance term that captures much of the variance in the Aß deposition pattern. We have previously demonstrated the utility of this model for human data from the Alzheimer's Disease Neuroimaging Initiative (ADNI). The objective of this study was to validate ESM in a transgenic mouse model of Alzheimer's disease. Methods 3D quantitative immunohistochemistry volumes from 12 (N=12, 4 male) and 19 (N=13, 6 male) month- old transgenic mutant human amyloid precursor protein (hAPP) mice were spatially normalized to an anatomical template. Mean values of Aß deposition were then automatically extracted from 25 gray matter regions. Connectivity between the 25 regions was estimated from publicly-available tracer studies (http://connectivity.brain-map.org/) and diffusion MRI data acquired from young, wild-type mice. An effective anatomical distance between each of pair regions i and j ($Edist_{i,j}$) was calculated as the shortest path between them (*i.e.* the more connected *i* and *j*, the lower the $Edist_{i,j}$ value). Using an iterative procedure, we then identified the epicenter region *i* that maximized the non-linear correlation between regional Aß deposition value and $Edist_{i,j}$ (j = 1 to 25). Results ESM identified posterior cingulate cortex (PCC) as the epicenter region, and the concentration of Aß increased with distance from PCC. Furthermore, the ESM model explains greater than 60% of the Aß patterns in hAPP mice at 12 and 19 months-of-age. Conclusions The strong correlation between effective anatomical distance and Aß deposition pattern supports the network-driven hypothesis of misfolded proteins propagation. The epicenter region that we identified, PCC, is a central component of the default mode network, and in humans has been linked to the progression of Alzheimer's disease. While explaining 60% of the Aß pattern is comparable to the best results obtained from human studies,

we expect this result will be improved in our mouse model by incorporating additional measures, such as cerebral blood flow and astrocytic/microglial density, in the model. Improved characterization of Aß deposition will provide us with a greater understanding of disease progression. (2167 - Needs to be no more than 2300 characters)

Disclosures: **B.J. Bedell:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biospective Inc.. **K. DeDuck:** None. **Y. Iturria-Medina:** None. **A.C. Evans:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biospective Inc..

Nanosymposium

207. Imaging the Healthy and the Diseased Brain

Location: 150A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 207.09

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH Grant NS075321

NIH Grant NS41509

NIH Grant NS48924

APDA Center for Advanced PD Research at Washington University

Greater St Louis Chapter of the APDA

McDonnell Center for Higher Brain Function

Hartke Fund

Title: Development of neuroimaging biomarkers for Parkinson disease: Pitfalls & potential

Authors: J. S. PERLMUTTER, *S. M. MOERLEIN;
Mallinckrodt Inst. of Radiology, Washington Univ., SAINT LOUIS, MO

Abstract: Molecular imaging with positron-emitting biomarkers has great potential for Parkinson disease research. Two major goals are to develop such biomarkers for objective measurement of Parkinson disease (PD) severity and to investigate disease pathophysiology.

Clinical trials have used a variety of molecular imaging biomarkers to quantify presynaptic dopaminergic neurons in the striatum. However, these studies report discordant results when using these neuroimaging biomarkers compared to clinical measures of disease progression. This failure may, in part, be due to inadequate preclinical validation of molecular imaging methods. Measurements of striatal and midbrain uptake of the biomarkers 6-[^{18}F]fluorodopa (FD; primarily reflects decarboxylase and storage), [^{11}C]dihydrotetrabenazine (DTBZ; reflects VMAT2) and 2β-[^{11}C]carbomethoxy-3β-4-fluorophenyltropane (CFT; reflects DAT) compared to motor behavior, nigral neuronal counts, and other in vitro striatal measures reveals a likely cause of these discordant findings. Furthermore, comparison of PET data using the Aβ amyloid biomarker [^{11}C]Pittsburgh compound B (PiB) in PD subjects to postmortem findings reveals the importance of such assessments for meaningful interpretation of the PET findings, and better understanding of the pathophysiology of dementia associated with PD. These examples underscore the essential prerequisite of careful validation of neuroimaging methods with biomarkers prior to clinical application.

Disclosures: J.S. Perlmutter: None. **S.M. Moerlein:** None.

Nanosymposium

207. Imaging the Healthy and the Diseased Brain

Location: 150A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 207.10

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: MJ Fox Foundation

Kinetics Foundation

Title: Utilization of different molecular weight magnetic resonance tracers for tracking GDNF distribution following convection enhanced delivery

Authors: *L. H. BRADLEY[1,2], C. ROSS[4], P. MARGAIRAZ[5], R. VENUGOPALAN[6], Z. ZHANG[1], P. A. HARDY[1,3];
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Abstract: Convection enhanced delivery (CED) is a powerful application for the targeted delivery of therapeutic compounds directly to the brain. However, without the use of magnetic resonance (MR) imaging, the volume of distribution (Vd) and targeting of the agents delivered through CED is not known in real time, thereby complicating the assessment of the drug's efficacy. Some researchers have attempted to infer the CED distribution of a therapeutic compound through the imaging of gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA), a MR-sensitive tracer. Although this compound has many advantages, it may not accurately approximate the distribution of therapeutic compounds that are much larger and have very different chemical properties, including non-specific binding. Our objective was to test the MW dependence of the MR tracer molecule distribution in vitro and in vivo, and to determine a tracer that would better represent the distribution of glial cell line-derived neurotrophic factor (GDNF), a neurotrophic factor evaluated for the treatment of Parkinson's disease (PD), when infused via CED. We used MR imaging of Gd-DTPA-tagged polylysine compounds of various MW, in vitro and in vivo, to measure the dependence of a compound's CED distribution on its MW. For the in vitro studies, the correlation between Vd in 0.6% agarose gels delivered through a multiport catheter as a function of MW was determined through MRI by measuring the T1 of the infused tracers. The compounds distributed in the gels inversely proportional to their MW, consistent with convection and diffusion according to the Einstein relationship. The same compounds were tested in vivo by infusing them into the putamen of non-human primates (NHP). While the Gd-DTPA distributed freely, the higher MW polylysine compounds did not suggesting that Vd of the tracer was impeded by non-specific binding to the brain's extracellular matrix. To compare the Vd of tracers of different MW and GDNF, we selected Gd-DTPA-albumin as a tracer that approximates the MW of GDNF, but lacks the non-specific binding properties of Gd-polylysine. Eight NHPs were co-infused with GDNF and either Gd-DTPA or Gd-DTPA-albumin and real time images were acquired during the infusion to monitor CED distribution. Images revealed a smaller distribution of the higher MW tracer, Gd-DTPA-albumin. Post-mortem immunohistochemistry revealed the distribution of GDNF was in close agreement to the Gd-DTPA-albumin. Collectively, our findings suggest that the use of tracers that match the molecular parameters of the therapeutic will more accurately indicate the CED distribution of therapeutic compounds.

Disclosures: L.H. Bradley: None. **C. Ross:** None. **P. Margairaz:** None. **Z. Zhang:** None. **P.A. Hardy:** None. **R. Venugopalan:** None.

Nanosymposium

207. Imaging the Healthy and the Diseased Brain

Location: 150A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 207.11

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Interrogating non-dopaminergic striatal systems in parkinson's: Pet imaging of pde10 and adenosine 2a

Authors: J. P. SEIBYL, *G. D. TAMAGNAN, D. JENNINGS, K. MAREK;
IND, NEW HAVEN, CT

Abstract: Imaging striatal presynaptic dopaminergic targets in Parkinson's (PD) has been useful for diagnostic assessment, but remains controversial for monitoring disease progression as underlying pathophysiologic processes entail more than nigrostriatal dopamine cell loss. Changes in regulation of the medium spiny neuron has been of particular interest owing to the central gatekeeping role these cells serve in regulating network traffic through striatal motor pathways. A number of neurochemical systems are involved in these pathways, offering targets for therapeutic intervention in movement disorders. Treatments focusing on the adenosine 2a (A2a) receptor and PDE10 are being evaluated in PD and Huntington's (HD). We have developed high affinity, selective 18F-labelled PET radiotracers for assessing in vivo A2a (MNI444) and PDE10 (MNI659), including performing PET biodistribution and dosimetry, pharmacokinetic modeling for optimized imaging outcome measures, and test retest reproducibility studies in human. Both tracers have been evaluated in movement disorders patients. [18F]MNI-659 is a promising striatal imaging biomarker, potentially capable of assessing the extent of disease in early manifest HD. Furthermore, [18F]MNI-659 may potentially identify early changes in medium spiny neurons and serve as a marker to predict conversion to manifest HD. [18F]MNI-444 has excellent potential as a PET radiotracer for imaging A2a receptors in human that can be non-invasively quantified. This tracer may be useful in elucidating striatal pathology in PD and HD and in evaluating receptor occupancy of A2a targeted therapies.

Disclosures: **J.P. Seibyl:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Molecular Neuroimaging,LLC. **G.D. Tamagnan:** A. Employment/Salary (full or part-time):; Molecular Neuroimaging,LLC. **D. Jennings:** A. Employment/Salary (full or part-time):; Molecular Neuroimaging,LLC. **K. Marek:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Molecular Neuroimaging,LLC.

Nanosymposium

207. Imaging the Healthy and the Diseased Brain

Location: 150A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 207.12

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: CIHR

Michael J. Fox Foundation

Pacific Alzheimer Research Foundation

Pacific Parkinson's Research Institute

Canada Research Chairs

National Parkinson Foundation

Title: Imaging neurodegeneration in Parkinson’s disease: Dopamine and beyond

Authors: *A. STOESSL;
Pacific Parkinson's Res. Ctr., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Parkinson’s disease (PD) has traditionally been considered a disorder of dopamine (DA) deficiency. Functional imaging studies with positron emission tomography (PET) or single photon emission computed tomography (SPECT) can quantitatively assess DA dysfunction using tracers directed towards the DA transporter (DAT), vesicular monoamine transporter type 2 (VMAT2) or L-aromatic amino acid decarboxylase (L-AAAD). Studies with any of these tracers reveal a reduction of tracer uptake with the following characteristics: (i) asymmetric; (ii) a gradient of dysfunction from caudal to rostral striatum; (iii) broad correlation between tracer uptake and clinical function; (iv) a time course of decline best described by an exponential function towards a non-zero asymptote, in which the rostral-caudal gradient is preserved . Challenges include the possibility of compensatory changes and effects of medication on the expression of DA markers, the lack of clear correlation between change in imaging marker expression and change in clinical measures. DA imaging can detect changes prior to symptom onset in subjects at high risk of PD and the trajectory of decline parallels that predicted by extrapolating back in patients with established disease. While not neurochemically specific, studies of glucose metabolism reveal networks of altered activity that differentiate between PD and atypical parkinsonian conditions and that progress over time. Several questions remain: (i) Is loss of DA function the optimum measure of disease progression in PD? (ii) What is the trajectory of impairment in non-DA systems? (iii) Does the pattern of impairment in early disease predict the future development of disease and treatment-related complications? (iv) What are the time course and distribution of neuroinflammatory changes in PD? The ability to assess

the distribution and degree of pathology, including the deposition of α-synuclein and other interacting proteins, would represent an enormous advance in the field, both for understanding the pathogenesis of disease, as well as the capacity to assess the effects of disease modifying therapies.

Disclosures: A. Stoessl: None.

Nanosymposium

207. Imaging the Healthy and the Diseased Brain

Location: 150A

Time: Sunday, November 16, 2014, 1:00 PM - 4:15 PM

Presentation Number: 207.13

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Alpha-synuclein SMART Molecule: a novel blood-brain barrier permeable diagnostic ligand for parkinson's disease

Authors: *A. CARTIER, R. BHATT;
ICB International, Inc., La Jolla, CA

Abstract: Development of imaging tracers for live visualization of pathologic proteins implicated in Parkinson's disease (PD) would be a game-changing achievement for the PD field. The ability to image such proteins in the brain provides an invaluable tool for diagnosis and assessment of drug therapy efficacy. A major impediment in developing imaging tracers for the central nervous system (CNS) is the blood-brain barrier (BBB) which prevents most molecules from entering the brain. Furthermore, target selectivity plays a crucial role in developing a highly specific ligand for imaging. Current in vivo imaging approaches for PD lack the ability to specifically visualize proteins involved in the pathology of this disorder early on in the course of disease, and monitor disease progression during therapy. SMART Molecules (SMs) were developed to deliver biologics across the BBB to specific targets within the CNS. Our SM approach utilizes the target specificity of conventional antibodies, and BBB permeability of small molecule drugs to create novel biologics ideal for in vivo imaging. We have generated a SMART Molecule for alpha-synuclein (α-syn-SM) since α-syn is a major pharmaceutical target for PD as its progressive accumulation, deposition and aggregation is a pathological hallmark of this disorder. Alpha-syn-SM demonstrates superior blood-brain penetration compared to conventional antibodies, and exquisite specificity for α-syn. Use of α-syn-SM as an imaging agent will allow specific monitoring of α-syn, and elucidating its temporal expression, topographical distribution and spatial relationship relative to disease pathology and clinical

changes that occur during disease progression. In this study, the unique trafficking and specificity of α-syn-SM to detect α-syn in the CNS of mice was demonstrated. We administered a single dose of Iodine-125 (125I) radio-labeled α-syn-SM (125I-α-syn-SM) into the tail-vein of non-transgenic (ntg) and PD-like transgenic (tg) mice. Whole body scans of mice were then obtained by single photon emission tomography (SPECT) imaging. Analysis of SPECT images revealed 125I-a-syn-SM uptake into the brain as early as one hour post injection with continued accumulation in the brain of transgenic mouse for at least 9 days. Biodistribution analysis of 125I-a-syn-SM showed 15% higher radioactivity counts in the brain compared to the blood in the tg mice. To the best of our knowledge, there are no published reports on an imaging tracer for α-syn demonstrating such BBB permeation kinetics with high target specificity. These results strongly support the application of a-syn SMART Molecule as a SPECT/PET ligand for Parkinson's disease.

Disclosures: A. Cartier: None. **R. Bhatt:** None.

Nanosymposium

282. Parkinson's Disease: LRRK2

Location: 147A

Time: Monday, November 17, 2014, 8:00 AM - 10:45 AM

Presentation Number: 282.01

Topic: C.03. Parkinson’s Disease

Support: Michael J. Fox Foundation for Parkinson's Disease

Title: Role of lrrk2 in human monocytes and t-cell subset frequencies and activation as a function of age as a potential contributor to immune dysfunction in idiopathic parkinson’s disease

Authors: *M. G. TANSEY[1], D. A. COOK[1], G. T. KANNARKAT[1], K. P. MACPHERSON[1], L. M. BUTKOVICH[1], J. CHANG[1], J. CHUNG[1], S. FACTOR[2], J. M. BOSS[3];
[1]Physiol., [2]Neurol. and Movement Disorders Ctr., [3]Microbiology and Immunol., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Leucine rich repeat kinase 2 (LRRK2) has high expression in cells of the immune system (CD4+ and CD8+ T cells, CD14+ monocytes, and CD19+ B cells), but its function in the immune system and its effects on age-related neurodegeneration are largely unknown. As individuals age, they become more susceptible to infections and the development of serious chronic diseases such as diabetes, cancer, Alzheimer’s and Parkinson’s disease. The immune

system functions to prevent and fight infection as well as repair damaged tissues. Over time, a phenomenon known as immunosenescence occurs, where immune function becomes dysregulated leading to both a persistent low level inflammation and an increased susceptibility to development of disease. LRRK2 is a protein highly expressed in immune cell that has been reported as a negative regulator of nuclear factor of activated T cells (NFAT), a transcription factor essential to proper T cell function and activation. Mutations or polymorphisms in the *lrrk2* gene are associated with many immunological diseases, including Parkinson's disease (PD), Crohn's disease, and increased risk for leprosy infection, indicating that it plays an important role in proper immune function. It is not currently known how LRRK2 expression changes with age. Studies are in progress to test the hypothesis that age-dependent changes in LRRK2 expression correlate to alterations in immune cell subset frequencies/function and risk for development of age-related diseases, in particular PD. We are immunophenotyping three separate age groups (25-44, 45-65, and 66-85) and quantifying the frequencies and activation states of monocytes and T cell subsets. Expression levels of LRRK2 will be quantified and correlated with immunophenotype data. By determining the role LRRK2 plays in modulating immune function, we will reveal opportunities to develop new therapies to treat or slow progression of age-related diseases involving immune cell dysfunction. [Funding support from the Michael J. Fox Foundation for Parkinson's Research *LRRK2 Role in Idiopathic PD* Program].

Disclosures: **M.G. Tansey:** None. **D.A. Cook:** None. **G.T. Kannarkat:** None. **K.P. MacPherson:** None. **L.M. Butkovich:** None. **J. Chang:** None. **J. Chung:** None. **S. Factor:** None. **J.M. Boss:** None.

Nanosymposium

282. Parkinson's Disease: LRRK2

Location: 147A

Time: Monday, November 17, 2014, 8:00 AM - 10:45 AM

Presentation Number: 282.02

Topic: C.03. Parkinson's Disease

Support: Michael J Fox Research Grant

American Parkinson Disease Association

Title: Lrrk2-mediated neuroinflammation in α-synuclein induced neurodegeneration

Authors: *J. LIMA DAHER, L. A. VOLPICELLI-DALEY, J. P. BLACKBURN, M. S. MOEHLE, A. B. WEST;
Neurol., Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Mutations in LRRK2 can cause late-onset Parkinson's disease (PD), but the pathophysiological role of LRRK2 in neurodegeneration is not clear. Recently, LRRK2 knockout rats have been characterized that might aid in understanding the function of LRRK2 in models of disease. LRRK2 knockout rats might also be useful for interpretation of LRRK2 as a therapeutic target for LRRK2-inhibitor based strategies. Here, we report LRRK2 knockout rats as resistant to dopaminergic (TH+ cells) neurodegeneration elicited by intracranial administration of highly purified preparations of LPS. Such resistance to dopaminergic neurodegeneration correlated well with reduced pro-inflammatory myeloid cell (CD68+ cells) recruitment to the midbrain. Moreover**,** adeno-associated virus mediated transduction of human α-synuclein also resulted in dopaminergic neurodegeneration in wild-type (WT) rats, whereas LRRK2 knockout (KO) animals had no significant loss of TH+ neurons as well as reduced numbers of pro-inflammatory CD68+ myeloid cells recruited to the SNpc. Although LRRK2 expression in the rat midbrain remained undetected under non-pathological conditions, LRRK2 became highly expressed in pro-inflammatory CD68/Iba1+ myeloid cells recruited to the SNpc in response to LPS exposure and α-synuclein over-expression. These results suggest that knocking down LRRK2 may protect from overt cell loss by inhibiting the recruitment of chronically activated pro-inflammatory myeloid cells. Therefore, LRRK2 inhibition may therefore be a potentially efficacious approach to slow or stop the progression of diseases of the brain where chronic myeloid cell activation drives aspects of dysfunction and loss of neurons, such as in Parkinson's disease.

Disclosures: J. Lima Daher: None. **L.A. Volpicelli-Daley:** None. **J.P. Blackburn:** None. **M.S. Moehle:** None. **A.B. West:** None.

Nanosymposium

282. Parkinson's Disease: LRRK2

Location: 147A

Time: Monday, November 17, 2014, 8:00 AM - 10:45 AM

Presentation Number: 282.03

Topic: C.03. Parkinson's Disease

Support: DZNE

Hertie Foundation

Title: Interferon gamma induces leucine-rich repeat kinase LRRK2 via extracellular signal-regulated kinase ERK5 in macrophages

Authors: M. KUSS, E. ADAMOPOULOU, *P. J. KAHLE;
HIH Dept. Neurodegeneration, Univ. of Tuebingen, Tuebingen, Germany

Abstract: The gene encoding leucine-rich repeat kinase 2 (LRRK2) comprises a major risk factor for Parkinson's disease. Recently, it has emerged that LRRK2 plays important roles in the immune system. LRRK2 is induced by interferon-gamma (IFNg) in monocytes, but the signaling pathway is not known. Here, we show that IFN-g-mediated induction of LRRK2 was suppressed by pharmacological inhibition and RNA interference of the extracellular signal-regulated kinase 5 (ERK5). This was confirmed by LRRK2 immunostaining, which also revealed that the morphological responses to IFN-g were suppressed by ERK5 inhibitor treatment. Both human acute monocytic leukemia THP-1 cells and human peripheral blood monocytes stimulated the ERK5-LRRK2 pathway after differentiation into macrophages. Thus, LRRK2 is induced via a novel, ERK5-dependent IFN-g signal transduction pathway, pointing to new functions of ERK5 and LRRK2 in human macrophages.

Disclosures: **M. Kuss:** None. **P.J. Kahle:** None. **E. Adamopoulou:** None.

Nanosymposium

282. Parkinson's Disease: LRRK2

Location: 147A

Time: Monday, November 17, 2014, 8:00 AM - 10:45 AM

Presentation Number: 282.04

Topic: C.03. Parkinson's Disease

Support: NIH Grant R01NS06493

NIH Grant U18NS082132

NIH Grant K02NS080915

Title: pSer1292 in the pathophysiology of Parkinson's disease

Authors: *K. B. FRASER[1], M. MOEHLE[1], J. BLACKBURN[1], R. ALCALAY[2], D. STANDAERT[1], A. WEST[1];
[1]Neurol., Univ. of Alabama At Birmingham, Birmingham, AL; [2]Columbia Univ., New York, NY

Abstract: The leucine-rich repeat kinase 2 (LRRK2) gene is of the most common known genetic causes of late-onset PD, albeit with a variable age-dependent penetrance for the most common mutation G2019S. Clinically carriers of LRRK2 mutation are similar to idiopathic PD, and most (but not all) autopsies of mutation carriers with PD demonstrated Lewy body pathology similar to idiopathic PD. The mechanism linking LRRK2 G2019S mutations to PD is unknown, but it is hypothesized that the mutation results in gain of function of kinase activity. Recently, an autophosphorylation site (pS1292) has been discovered and can be measured both *in vitro* and *in vivo.* We show uniformly in recombinant protein derived from transfected cells that pathogenic mutations up-regulate the proportion of pS1292 to total LRRK2. When this ratio is combined with pS935 to total LRRK2 ratios, some pathogenic mutations separate from wild-type LRRK2 by several orders of magnitude. We hypothesize that p1292-LRRK2 might demarcate the active form of the LRRK2 enzyme. Biochemical studies performed in cell lines, in tissue from rodents, as well as in clinical samples, may help reveal whether LRRK2 activity is altered in certain cellular conditions or diseased states, and be useful for both predictions of PD onset and severity and as a biomarker for LRRK2 inhibition.

Disclosures: K.B. Fraser: None. **M. Moehle:** None. **J. Blackburn:** None. **R. Alcalay:** None. **D. Standaert:** None. **A. West:** None.

Nanosymposium

282. Parkinson's Disease: LRRK2

Location: 147A

Time: Monday, November 17, 2014, 8:00 AM - 10:45 AM

Presentation Number: 282.05

Topic: C.03. Parkinson’s Disease

Title: Transcriptional signature of LRRK2 genetic variants in human brain

Authors: H. RHINN[1], *A. ABELIOVICH[2];
[1]Pathology and Cell Biol., Columbia Univ., New York, NY; [2]Pathol, Neurol, Cnt Neurobio & Behav, Columbia Univ., NEW YORK, NY

Abstract: Recent genome-wide association studies have linked common variants in the human genome to Parkinson’s disease (PD) risk. We have recently shown that the consequences of variants at 2 such loci, PARK16 and LRRK2, are highly interrelated, both in terms of their broad impacts on human brain transcriptomes of unaffected carriers, and in terms of their genetic associations with PD risk. Further experimental validation confirmed the interaction between the PARK16 locus gene RAB7L1 and LRRK2. We have expanded the transcriptomic

characterization of the LRRK2 locus and its relationship with other loci in additional brain gene expression datasets. Such an unbiased transcriptomic characterization of the LRRK2 risk allele is leveraged to identify genes that are functionally related to LRRK2 or drugs modulating the pathway affected by the presence LRRK2 PD risk allele.

Disclosures: H. Rhinn: None. **A. Abeliovich:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; UCB. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alector. F. Consulting Fees (e.g., advisory boards); Alector.

Nanosymposium

282. Parkinson's Disease: LRRK2

Location: 147A

Time: Monday, November 17, 2014, 8:00 AM - 10:45 AM

Presentation Number: 282.06

Topic: C.03. Parkinson’s Disease

Support: NIH/NINDS P50NS038377

MDSCRF 2007-MSCRFI-0420-00

MDSCRF 2009-MSCRFII-0125-00

MDSCRF 2013-MSCRFII-0105-00

Title: Ribosomal protein phosphorylation in LRRK2-mediated neurodegeneration

Authors: *I. MARTIN;
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Abstract: A link between elevated LRRK2 kinase activity and neurodegeneration has been established, and identifying pathogenic LRRK2 substrates is important for understanding LRRK2 toxicity. We identified ribosomal protein s15 as an important LRRK2 substrate which underlies G2019S LRRK2 toxicity in Drosophila and human neuron PD models. Expression of phospho-deficient s15 substantially rescued G2019S LRRK2-mediated cell death in human dopamine neurons and blocked neurodegenerative phenotypes in G2019S LRRK2 transgenic Drosophila. Pathogenic LRRK2 was found to stimulate a bulk increase in protein synthesis in

flies that was dependent on s15 phosphorylation and consistent with an observed induction of cap-dependent and cap-independent translation by LRRK2 in vitro. Hence, s15 is a kinase substrate of LRRK2 which is pivotal to its toxicity and the effects of pathogenic LRRK2 on mRNA translation.

Disclosures: I. Martin: None.

Nanosymposium

282. Parkinson's Disease: LRRK2

Location: 147A

Time: Monday, November 17, 2014, 8:00 AM - 10:45 AM

Presentation Number: 282.07

Topic: C.03. Parkinson's Disease

Support: NIH ES15567

NIH NS060872

Howard Hughes Medical Institute

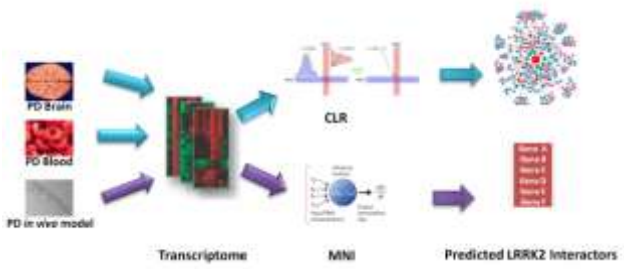
NIH NS072519

Michael J Fox Foundation

Title: A Parkinson's disease gene regulatory network identifies the signaling protein RGS2 as a modulator of LRRK2 activity and neuronal toxicity

Authors: *B. L. WOLOZIN[1], J. DUSONCHET[2], H. LI[3], M. GUILLILY[2], M. LIU[4], J. Y. BOON[2], A. MAMAIS[5], Z. YUE[6], R. BANDOPADHYAY[5], M. A. GLICKSMAN[4], D. J. MOORE[7], J. J. COLLINS[8];
[1]Pharmacol, Boston Univ. Schl Med., BOSTON, MA; [2]Pharmacol., Boston Univ. Sch. of Med., Boston, MA; [3]Mol. Pharmacol. and Systems Therapeut., Mayo Clin., Rochester, MN; [4]Lab. for Drug Discovery in Neurodegeneration, Brigham and Women's Hosp., Boston, MA; [5]Inst. of Neurol., Univ. College, Londong, London, United Kingdom; [6]Depts. of Neurol. and Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; [7]Ctr. for Neurodegenerative Sci., Van Andel Inst., Grand Rapids, MI; [8]Wyss Inst. for Biologically Inspired Engin., Harvard Univ., Boston, MA

Abstract: Mutations in LRRK2 are one of the primary genetic causes of Parkinson's disease (PD). LRRK2 contains a kinase and a GTPase domain, and familial PD mutations affect both enzymatic activities. Yet the signaling mechanisms underlying the regulation of LRRK2 kinase and GTPase activities and the pathogenic effects of familial mutations remain unknown. Identifying the signaling proteins that regulate LRRK2 function and toxicity remains a critical goal for the development of effective therapeutic strategies. In this study, we apply systems biology tools to human PD brain and blood transcriptomes to reverse-engineer a LRRK2-centered gene regulatory network. This network identifies several putative master regulators of LRRK2 function. In particular, the signaling gene RGS2, which encodes for a GTPase-activating protein (GAP), is a key regulatory hub connecting the familial PD-associated genes DJ-1 and PINK1 with LRRK2 in the network. RGS2 expression levels are reduced in the striata of LRRK2 and sporadic PD patients. We identify RGS2 as a novel interacting partner of LRRK2 in vivo. RGS2 regulates both the GTPase and kinase activities of LRRK2. We show in mammalian neurons that RGS2 regulates LRRK2 function in the control of neuronal process length. RGS2 is also protective against neuronal toxicity of the most prevalent mutation in LRRK2, G2019S. We find that RGS2 regulates LRRK2 function and neuronal toxicity through its effects on kinase activity and independently of GTPase activity, which reveals a novel mode of action for GAP proteins. This work identifies RGS2 as a promising target for interfering with neurodegeneration due to LRRK2 mutations in PD patients.



Disclosures: **B.L. Wolozin:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aquinnah Pharmaceuticals Inc.. **J. Dusonchet:** None. **H. Li:** None. **M. Liu:** None. **J.Y. Boon:** None. **A. Mamais:** None. **Z. Yue:** None. **R. Bandopadhyay:** None. **M.A. Glicksman:** None. **D.J. Moore:** None. **J.J. Collins:** None. **M. Guillily:** None.

Nanosymposium

282. Parkinson's Disease: LRRK2

Location: 147A

Time: Monday, November 17, 2014, 8:00 AM - 10:45 AM

Presentation Number: 282.08

Topic: C.03. Parkinson's Disease

Support: NIH/NINDS P50NS038377

Title: A novel mouse model of Parkinson's disease with conditional expression of mutant LRRK2 in dopaminergic neurons causes progressive neurodegeneration

Authors: *Y. XIONG[1], X. MAO[1,4], J. N. STANKOWSKI[1,4], B. LEE[1], H. KO[1,5], Y. LEE[1,4], S. NEIFERT[1], J. C. GRIMA[2], D. SWING[6], L. IACOVITTI[7], L. TESSAROLLO[6], T. M. DAWSON[1,4,2], V. L. DAWSON[1,4,2,3];
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Abstract: Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene have been identified as an unambiguous cause of late-onset, autosomal dominant familial Parkinson's disease (PD). LRRK2 is the strongest genetic risk factor in sporadic PD known to date. To model LRRK2-associated PD, a number of transgenic mice expressing wildtype or mutant LRRK2 have been described with varying degrees of pathology and LRRK2-related abnormalities. However, none of these models fully recapitulated PD pathogenesis, and particularly none of them exhibited a progressive loss of dopaminergic (DA) neurons with aging. The distinct lack of progressive nigrostriatal DA degeneration in the current LRRK2 transgenic mouse models is likely due to the promoters used for transgene expression, as none of them drives LRRK2 expression selectively in DA neurons. Accordingly, to selectively express human LRRK2 within DA neurons, we developed a novel human tyrosine hydroxylase-controlled tetracycline transactivator (hTH-tTA) mouse driver line that can specifically drive transgene expression at high levels in the nigrostriatal pathway. Using this driver line, we developed a novel LRRK2 mutant G2019S (GS) transgenic mouse line and a corresponding functionally-negative control G2019S/D1994A (GS/DA) mouse line. Together with a binary tetracycline-dependent inducible gene expression system, we can selectively induce high expression of GS LRRK2 or the control GS/DA LRRK2 in DA neurons. Markedly selective expression of LRRK2 in the nigrostriatal pathway leads to progressive DA neuron degeneration in the substantia nigra pars compacta (SNpc) and behavioral defects with aging. As such, our novel LRRK2 transgenic mouse models provide robust tools for understanding the mechanisms of LRRK2-induced DA neurotoxicity *in vivo*, as well as a valuable platform for disease analysis and drug development.

Disclosures: **Y. Xiong:** None. **X. Mao:** None. **J.N. Stankowski:** None. **B. Lee:** None. **H. Ko:** None. **Y. Lee:** None. **S. Neifert:** None. **J.C. Grima:** None. **D. Swing:** None. **L. Iacovitti:** None. **L. Tessarollo:** None. **T.M. Dawson:** None. **V.L. Dawson:** None.

Nanosymposium

282. Parkinson's Disease: LRRK2

Location: 147A

Time: Monday, November 17, 2014, 8:00 AM - 10:45 AM

Presentation Number: 282.09

Topic: C.03. Parkinson's Disease

Support: MOST 102-2314-B-002-111-MY3

NSC 98-2628-B-002-072-MY3

Title: Mutation of LRRK2 ROC domain induces motor dysfunction, neuronal loss and autophagy in a transgenic moouse model

Authors: ***M.-L. CHEN**[1,2], R.-M. WU[1];
[1]Dept. of Neurol., Natl. Taiwan Univ. Hosp., Taipei, Taiwan; [2]Dept. of Life Sci., Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disorder by selective loss of dopaminergic neurons in the substantia nigra and the formation of intracellular α-synuclein (α-syn) inclusions in Lewy bodies within the basal ganglia. Genetic variability in leucine-rich repeat kinase 2 (LRRK2) is the most common genetic causes of sporadic and familial PD. Although pathologic R1441C/G mutants on ROC domain reduce the rate of GTP hydrolysis and increase the kinase activity and GTP-binding activity and then cause cell death in vitro, how they drive neuropathology is critical to understanding the mechanisms of PD. To study the pathogenic role of LRRK2 ROC domain in PD, we report a detailed behavioural characterization of several human LRRK2 (hLRRK2) transgenic mouse lines where high-levels of mutant (heterozygous and homozygous R1441G) and wild type (WT) hLRRK2 compared to littermate control mice focused on motor phenotypes. R1441G heterozygous and homozygous hLRRK2 mice weighed significantly less than age-matched non-transgenic and WT hLRRK2 mice. The behavioral analyses reveal that WT hLRRK2 mice reduced the fine frequencies of locomotor activity in peripheral field, compared to age-matched non-transgenic mice. However, R1441G homozygous hLRRK2 increase the fine frequencies of locomotor activity in center field and diminished the ambulatory ambulatory frequencies of locomotor activity in peripheral

fieldand the forelimb grip strength, compared to age-matched non-transgenic mice. In addition, the rear activity of R1441G and WT hLRRK2 animals were less than age-matched non-transgenic mice. Using immunohistochemical, we observe moderate, progressive DAergic neurodegeneration in the substantia nigra pars compacta (SNpc) of the R1441G hLRRK2 animals. Compared to the age-matched non-transgenic mice, we initially observe the loss of DAergic fiber density in the SN pars reticulata (SNr), followed by the loss of DA neurons in SNpc. Moreover, the R1441G hLRRK2 mice have an age-dependent increase in LC3 expression, an autophagy marker in the SNpc. Our results suggest that autophagy plays a role in mutant hLRRK2-dependent neurodegeneration.

Disclosures: **M. Chen:** None. **R. Wu:** None.

Nanosymposium

282. Parkinson's Disease: LRRK2

Location: 147A

Time: Monday, November 17, 2014, 8:00 AM - 10:45 AM

Presentation Number: 282.10

Topic: C.03. Parkinson’s Disease

Support: NINDS NS065860

NINDS NS40256,

NINDS NS072187

NIEHS ES020715

Michael. J. Fox

Title: Progressive dopaminergic alterations and mitochondrial abnormalities in LRRK2 G2019S knock in mice

Authors: M. YUE[1], K. HINKLE[1], P. DAVIES[2], F. FIESEL[1], E. TRUSHINA[3], T. CHRISTENSEN[3], E. BOWLES[1], B. BEHROUZ[1], S. LINCOLN[1], J. BEEVERS[1], A. MILNERWOOD[4], A. KURTI[1], J. FRYER[1], W. SPRINGER[1], D. DICKSON[1], M. FARRER[4], *H. L. MELROSE[1];
[1]Mayo Clin. Jacksonville, Jacksonville, FL; [2]MRC Protein Phosphorylation Unit, Dundee, United Kingdom; [3]Mayo Clin. Rochester, Jacksonville, MN; [4]Ctr. for Applied Genetics,, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Mutations in the LRRK2 gene represent the most common genetic cause of late onset Parkinson's disease. The physiological and pathological roles of LRRK2 are yet to be fully determined but evidence points towards LRRK2 mutations causing a gain in kinase function, impacting on neuronal maintenance, vesicular dynamics and neurotransmitter release. To explore the role of physiological levels of mutant LRRK2, we created knock in mice harboring the most common LRRK2 mutation G2019S in their own genome. We have performed comprehensive dopaminergic, behavioral and neuropathological analyses in this model up to 24 months of age. We find elevated kinase activity in the brain of both heterozygous and homozygous mice. Although normal at 6 months, by 12 months of age, basal and pharmacologically-induced extracellular release of dopamine is impaired in both heterozygous and homozygous mice, corroborating previous findings in transgenic models over-expressing mutant LRRK2. Via measurement of basal and drug- evoked extracellular release of dopamine and its metabolites, our findings indicate that exocytotic release from the vesicular pool is impaired. Initial data also suggests that this release deficit can be rescued with a specific brain penetrant LRRK2 inhibitor. While no overt Parkinson's hallmark pathology is observed in aged knock-in mice, profound mitochondrial abnormalities are evident in the striatum of older homozygous G2019S mice, including the presence of condensed and misshapen mitochondria and alterations in mitochondrial fission proteins Drp-1 and Fis-1. We anticipate the G2019S will be a useful pre-clinical model for further evaluation of early mechanistic events in LRRK2 pathogenesis and for second-hit approaches to model disease progression.

Disclosures: **M. Yue:** None. **K. Hinkle:** None. **P. Davies:** None. **F. Fiesel:** None. **E. Trushina:** None. **T. Christensen:** None. **E. Bowles:** None. **B. Behrouz:** None. **S. Lincoln:** None. **J. Beevers:** None. **A. Milnerwood:** None. **A. Kurti:** None. **J. Fryer:** None. **W. Springer:** None. **D. Dickson:** None. **M. Farrer:** None. **H.L. Melrose:** None.

Nanosymposium

282. Parkinson's Disease: LRRK2

Location: 147A

Time: Monday, November 17, 2014, 8:00 AM - 10:45 AM

Presentation Number: 282.11

Topic: C.03. Parkinson’s Disease

Support: NIH - 5 R01-NS072359

Title: Plasticity in the hippocampus of LRRK2 mutant mice

Authors: *E. S. SWEET[1], B. SAUNIER-REBORI[2], Z. YUE[1], R. D. BLITZER[2];
[1]Neurol., [2]Pharmacol. and Systems Therapeut., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Parkinson's disease (PD) is a major movement disorder characterized pathologically by the loss of the dopamine neurons and formation of Lewy bodies. In addition to motor abnormalities, PD patients display a variety of non-motor symptoms, including executive function and memory loss. These non-motor symptoms are not treated well by current dopamine replacement or brain stimulation therapies. In order to explore the mechanisms that produce these symptoms we used a BAC transgenic mouse model of the PARK8 gene encoding leucine-rich repeat kinase (LRRK2). Mutations in the LRRK2 gene are the most common genetic factors associated with PD; among these mutations is G2019S, which increases LRRK2 kinase activity. LRRK2 is highly expressed in brain regions that are involved with non-motor functions, including the frontal cortex and the hippocampus, and thus is likely to play a role in non-motor PD symptoms. We used electrophysiological method to study the pathogenic role that the LRRK2 G2019S mutation, associated with increased kinase activity, plays in Schaffer collateral-CA1 synapse of the dorsal hippocampus. In field and whole-cell patch recordings, we studied basal synaptic function and synaptically-induced forms of plasticity, including PPF, LTP and LTD. Our results show that an increase in kinase activity due to the LRRK2-G2019S mutation is associated with altered short- and long-term changes in synaptic function. We conclude that LRRK2 may play a role in normal hippocampal function and in some of the non-motor deficits seen in PD.

Disclosures: **E.S. Sweet:** None. **B. Saunier-Rebori:** None. **Z. Yue:** None. **R.D. Blitzer:** None.

Nanosymposium

283. Aging Brain and Cognition

Location: 152B

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 283.01

Topic: C.05. Aging

Support: PO1 AG22550

IAADR seed grant.

Title: Exercise but not antioxidants reversed age-related declines in motor function

Authors: *A. SIDHU, P. VANN, J. WONG, N. SUMIEN;
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Abstract: Aging has been associated with a decline in motor learning and performance. Interventions such as exercise and antioxidant supplementation when implemented independently have had a beneficial impact on motor function in both human and animal studies. While health conscious individuals will combine exercise with antioxidant consumption on the premise of added benefits, recent studies have indicated a potential for an antagonistic interaction of antioxidants with exercise. The current study aimed at determining the nature of the interaction between exercise and antioxidants on functional outcomes in young and old mice. Separate groups of young (4 months), and old (20 months) male C57BL/6J mice were assigned to one of the following treatments: sedentary/control diet (SedCon), sedentary/antioxidant-rich diet (vitamin E (100mg/kg/d) and vitamin C (200 mg/kg/d) (SedEC)), exercise/control diet (ExCon), exercise/antioxidant-rich diet (ExEC). After 8 weeks of pre-treatment, the mice underwent a series of behavioral tests, including spontaneous activity, coordinated running, bridge walking and tail suspension test, while remaining on their respective condition. Traveled distance was unaffected by treatments in the young mice, but was increased by all treatments equally in the old ones. ExCon and ExEC had higher latencies than SedCon and SedEC on the rotorod test, especially in the old mice. There was no effect of the treatments in young mice on the bridge walking test, however ExCon and ExEC had higher latencies that SedCon. In the tail suspension test, immobility time was decreased in ExCon and ExEC especially in the old mice. A moderate exercise regimen reversed age-related declined in motor function and improved anxiety level, while antioxidant supplementation was ineffective. There was no interaction between the two interventions on motor outcomes, either additive or antagonistic.

Disclosures: **A. Sidhu:** None. **P. Vann:** None. **J. Wong:** None. **N. Sumien:** None.

Nanosymposium

283. Aging Brain and Cognition

Location: 152B

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 283.02

Topic: C.05. Aging

Support: NIH AG025526

Title: Individual differences in aerobic fitness influence the regional pattern of brain volume in healthy aging

Authors: *G. E. ALEXANDER[1,2,7], M. C. FITZHUGH[3,7], D. A. RAICHLEN[4], K. A. HAWS[3,7], G. A. TORRE[3,7], T. P. TROUARD[5,7], G. A. HISHAW[6];
[1]Dept. of Psychology, Univ. of Arizona, TUCSON, AZ; [2]Neurosci. and Physiological Sci. Grad. Interdisciplinary Programs, [3]Dept. of Psychology, [4]Sch. of Anthrop., [5]Biomed. Engin., [6]Neurol., Univ. of Arizona, Tucson, AZ; [7]Evelyn F. McKnight Brain Inst., Tucson, AZ

Abstract: Individual differences in aerobic fitness levels may be an important factor affecting heterogeneity in brain aging and associated age-related cognitive decline. We recently proposed that increased demands for physical activity helped to support the evolution of long human lifespans and healthy brain aging (Raichlen and Alexander, Trends Neurosci, 2014). In this study, we sought to evaluate how individual differences in aerobic fitness levels effect regional brain volumes that are altered in the context of healthy aging. Quantitative measures of aerobic fitness (VO2max) during a graded exercise treadmill test were acquired in 155 healthy, community-dwelling adults, 50 to 89 years of age to determine whether those brain regions showing reductions in volume with increasing age are also altered by individual differences in VO2max. Participants (85F/70M; mean ± sd age = 69.6 ± 10.0 years; mean ± sd Mini-Mental State Exam = 29.0 ± 1.3) were medically screened to exclude neurological and psychiatric illnesses that could impact cognitive function. Regional patterns of brain volume were assessed using Freesurfer software with T1-weighted 3T volumetric magnetic resonance imaging (MRI) scans to identify the regional patterns of gray matter associated with age and VO2max. The results showed a regional pattern of gray matter reductions with increasing age that included bilateral superior, middle, and inferior frontal, superior and middle temporal, fusiform/lingual gyri, insula, inferior parietal, paracentral, cuneus, and precuneus regions (FDR correction, $p < 0.05$). After we controlled for the effects of aging in the sample, greater levels of VO2max were associated with greater volumes in distinct regions of bilateral medial frontal, anterior cingulate, lateral occipital, and entorhinal cortices. In addition, greater levels of VO2max were associated with increased volumes in several of the regions directly affected by aging in this cohort, including bilateral middle frontal, insula, fusiform/lingual gyri, and precuneus regions (FDR correction, $p < 0.05$). These findings provide initial support for a regionally distributed pattern of brain volume associated with individual differences in aerobic fitness levels in neurologically healthy middle-aged to elderly adults, suggesting that having higher levels of physical activity may help compensate for regional differences in gray matter volume often associated with brain aging.

Disclosures: G.E. Alexander: None. **M.C. Fitzhugh:** None. **D.A. Raichlen:** None. **K.A. Haws:** None. **G.A. Torre:** None. **T.P. Trouard:** None. **G.A. Hishaw:** None.

Nanosymposium

283. Aging Brain and Cognition

Location: 152B

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 283.03

Topic: C.05. Aging

Support: EY0212525

Nathan Shock Center for aging research

Fraternal Order of the Eagle

Jane B Cook

Title: Lifestyle choices to protect the aging brain - preserving Apoe expression and reversing neurovascular decline

Authors: *G. R. HOWELL, K. ONOS, L. GRAHAM, S. SIMEONE, I. SOTO;
The Jackson Lab., Bar Harbor, ME

Abstract: The high incidence of dementia in people of advanced age has become an important public health issue. Age-related cognitive decline and dementia negatively impact healthspan and longevity. The E4 allele of APOE (ApoE4) is the greatest genetic risk factor for decreased longevity and for the development of dementias such as Alzheimer's disease (AD). Deletion of APOE in the mouse leads to vascular and neuronal dysfunction (e.g. synapse loss) in the brain. In the brain, APOE is synthesized by astrocytes, which are important regulators of the neurovascular unit and maintain blood brain barrier (BBB) function, synaptic transmission and neural metabolism. Recent evidence suggests that in the aging brain astrocytes undergo a process of cellular senescence that can repress the capacity of these cells to support neural function. We have found that astrocyte expression of Apoe is downregulated in the neocortex of aging (18-24 months) mice when compared with young-adult mice (4-8 months). This change in Apoe expression was accompanied by pathological alterations in the microvascular structure that included uneven coverage of basement membrane along the vessels as a result of alterations to the basement membrane (BM) proteins. Loss and degeneration of pericytes were also observed by PDGFR-b immunoreactivity and transmission electron microscopy (TEM). These cerebrovascular pathologies, changes in BM and loss of pericytes, were also observed in adult (9 months) APOE-deficient mice suggesting an APOE-dependent breakdown of the neurovascular unit with age. Furthermore, exercise (voluntary running) but not diet restriction (DR) preserves neurovascular integrity and preserves Apoe RNA expression in brains of aged mice, suggesting a positive effect of exercise on astrocyte function and neurovascular integrity.

Disclosures: G.R. Howell: None. **I. Soto:** None. **K. Onos:** None. **L. Graham:** None. **S. Simeone:** None.

Nanosymposium

283. Aging Brain and Cognition

Location: 152B

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 283.04

Topic: C.05. Aging

Support: European Research Council (E.R.C.) grant given to M.S. (232835)

EU Seventh Framework Program (FP7) through the TargetBrain consortium given to M.S. (279017)

E.R.C. grant given to I.A. (309788)

Israeli Science Foundation grant given to I.A. (1782/11)

Frontiers Science Program Career Development Award given to I.A.

National Institute on Aging grant given to T.W.-C. (AG045034)

Jane Coffin Childs Postdoctoral fellowship (to J.M.C.)

Title: Aging-induced immunological response of the brain's choroid plexus negatively regulates neurogenesis and cognitive function

Authors: *K. BARUCH[1], A. DECZKOWSKA[1], E. DAVID[1], J. M. CASTELLANO[2], O. MILLER[1], A. KERTSER[1], T. BERKUTZKI[1], Z. ITZHAKI[1], D. BEZALEL[1], T. WYSS-CORAY[2], I. AMIT[1], M. SCHWARTZ[1];
[1]Weizmann Inst. of Sci., Rehovot, Israel; [2]Stanford Univ. Sch. of Med., Stanford, CA

Abstract: Multiple lines of evidence indicate that non-tissue-autonomous factors modulate brain senescence; however, the sources and roles of signals that shape brain’s function in the aged body remain enigmatic. Recent studies identified the brain's choroid plexus (CP), a ventricular epithelial structure which forms the blood-cerebrospinal fluid-barrier (BCSFB), as a site of continuous dialogue between the brain and blood-borne leukocytes. Here, we hypothesized that understanding of how CP function is regulated in aging may lead to identification of novel strategies to moderate age-associated decline in brain function. We performed multi-organ high-throughput transcriptome analysis of young and aged mice, and found that aging of the CP is characterized by a unique immunological signature, which we also found in aged human postmortem CPs. Uncoupling the aging process of the immune system from that of the brain, by using a surgical parabiosis model in which old mice shared vasculature with young mice, we

further found that this signature was not induced by the aged systemic milieu, but by brain-derived signals found in the cerebrospinal fluid of aged animals. Finally, neutralization of the CP-derived immunological signals within the brain's territory in aged mice led to partial restoration of cognitive function and hippocampal neurogenesis. Taken together, our results identify a persistent aging-induced response at the CP as a negative regulator of brain function, and thus suggest a novel target for therapeutic anti-aging intervention. K.B. and A.D. contributed equally to this study.

Disclosures: **K. Baruch:** None. **A. Deczkowska:** None. **E. David:** None. **J.M. Castellano:** None. **O. Miller:** None. **A. Kertser:** None. **T. Berkutzki:** None. **Z. Itzhaki:** None. **D. Bezalel:** None. **T. Wyss-Coray:** None. **I. Amit:** None. **M. Schwartz:** None.

Nanosymposium

283. Aging Brain and Cognition

Location: 152B

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 283.05

Topic: C.05. Aging

Support: Swedish Brain Foundation (Hjärnfonden)

Swedish Brain Power

Swedish Society for Medical Research (SSMF)

Foundation for Geriatric Diseases at Karolinska Institutet

ERC Advanced Investigator grant (322744)

Swedish Research Council

Alzheimer Foundation

Title: Germline mitochondrial DNA mutations can reduce lifespan

Authors: *J. M. ROSS[1], G. COPPOTELLI[1], B. J. HOFFER[2], L. OLSON[1];
[1]Dept. of Neurosci., Karolinska Institutet, Stockholm, Sweden; [2]Dept. of Neurosurg., Case Western Reserve Med. Ctr., Cleveland, OH

Abstract: Accumulation of mitochondrial DNA (mtDNA) mutations resulting in mitochondrial dysfunction has been heavily implicated in mitochondrial diseases as well as aging and age-related diseases, such as Alzheimer's and Parkinson's. Replication of the mitochondrial genome continues in mitotic and meiotic cells, as well as in non-dividing cells; thus, mutations can occur in the maternal germline and be transmitted to offspring. Knock-in mice expressing a proofreading deficient version of the nucleus-encoded catalytic subunit (*PolgA*) of mtDNA polymerase-γ were used to test the extent to which inherited mtDNA mutations can contribute to mitochondrial-driven aging. Homozygous $PolgA^{mut/mut}$ mice (mtDNA mutator mice) develop high levels of point mutations (20-30 mutations per mtDNA molecule) and linear deletions (25% of total mtDNA). The mtDNA mutator mice show many signs of premature ageing seen in humans, including reduced lifespan (42-43 wks), alopecia, weight loss, anemia, sarcopenia, loss of subcutaneous fat, reduced fertility, impaired hearing, and osteoporosis. Using these mutant mice, we recently reported (Ross et al. Nature, 2013) that ongoing mtDNA mutagenesis in the maternal germline causes anticipation of fecundity phenotypes when intercrossing heterozygotes. We also showed that germline mtDNA mutations can aggravate the premature aging phenotypes and lifespan in homozygous mtDNA mutator mice. Additionally, we discovered that 32% of mtDNA mutator mice exhibit stochastic disturbances of brain development, when maternal mtDNA mutations were combined with homozygosity for the *PolgA* mutation leading to de novo somatic mtDNA mutations. Surprisingly, we also find that maternally transmitted mtDNA mutations can cause mild premature aging phenotypes in mice with a wild-type nuclear DNA background, including alopecia, kyphosis, reduced body size, lower body weight, and decreased spontaneous rearing. These results suggest that starting life with healthy mitochondria is important for the maintenance of health during aging. We have followed the lifespan of these animals and have now discovered that not only do germline mtDNA mutations affect the onset and progression of aging phenotypes in these wild-type mice, but that longevity is also reduced by 30%. We thus present evidence to demonstrate that low levels of germline-transmitted mtDNA mutations per se can have life-long consequences not only by causing premature aging phenotypes, but also by shortening lifespan.

Disclosures: **J.M. Ross:** None. **G. Coppotelli:** None. **L. Olson:** None. **B.J. Hoffer:** None.

Nanosymposium

283. Aging Brain and Cognition

Location: 152B

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 283.06

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Pre-doctoral Training Grant 5T32 GM008541 (MW, RF)

PhRMA Foundation Pre-doctoral Fellowships (MW, RF)

Boston University UROP Scholarship (JB)

BU Faculty Recruit Fund (TI)

Title: miR-155/IL-6 axis regulates microglial inflammation-induced abnormality in neural differentiation in the dentate gyrus

Authors: *M. E. WOODBURY[1], R. W. FREILICH[2], J. D. BOUCHER[2], S. IKEZU[2], K. INGRAHAM[2], H. ASAI[2], C. J. CHENG[4], F. SLACK[4], T. IKEZU[3];
[1]Grad. Program in Neuroscience; Pharmacol. and Exptl. Therapeut., [2]Pharmacol. and Exptl. Therapeut., [3]Pharmacol. and Exptl. Therapeutics; Neurol., Boston Univ. Sch. of Med., Boston, MA; [4]Dept. of Molecular, Cell. and Developmental Biol., Yale Univ., New Haven, CT

Abstract: Neurodevelopmental psychiatric disorders, including Down syndrome (DS) and autism spectrum disorders (ASD), are estimated to affect over 2 million people in the U.S. and thus pose an immense burden to society. Neuroinflammation and abnormalities in neurogenesis are key features of these disorders, and animal models have demonstrated that pro-inflammatory challenge suppresses neurogenesis. A growing body of evidence implicates microRNAs (miRNAs) in regulation of the peripheral acute inflammatory response. To elucidate the influence of miRNAs on the acute CNS inflammatory response, primary murine microglia were stimulated with lipopolysaccharide (LPS), a pro-inflammatory Toll-Like Receptor 4 ligand, and profiled for gene and miRNA expression. miR-155 and interleukin-6 (IL-6) were the most up-regulated miRNA and mRNA identified in LPS-stimulated microglia when compared to control. Using *in vitro* and *in vivo* methods, we found that miR-155 critically regulates IL-6 gene induction in microglia under inflammatory challenge. In *in vitro* co-culture studies, LPS-stimulated microglia co-cultured with neural stem cells led to skewing towards gliogenesis, whereas blockade of IL-6 or genetic ablation of miR-155 in microglia restored neural differentiation. Moreover, nestin promoter-driven Cre recombinase-mediated expression of miR-155 in neural and hematopoietic stem cells led to striking abnormalities in arborization and localization of Doublecortin-positive immature neurons in the granular cell layer of the dentate gyrus. Our findings demonstrate that miR-155 regulates inflammation-induced neurogenic deficits as well as abnormal development of immature neurons, and reveal the miR-155/IL-6 axis as a novel therapeutic target for neurodevelopmental disorders.

Disclosures: M.E. Woodbury: None. **R.W. Freilich:** None. **J.D. Boucher:** None. **S. Ikezu:** None. **K. Ingraham:** None. **H. Asai:** None. **C.J. Cheng:** None. **F. Slack:** None. **T. Ikezu:** None.

Nanosymposium

283. Aging Brain and Cognition

Location: 152B

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 283.07

Topic: C.05. Aging

Support: Alzheimer's Research UK/Chief Scientist's Office, Scotland

University of Edinburgh Wellcome Trust Institutional Strategic Support Fund

Age UK (The Disconnected Mind project)

UK's Biotechnology and Biological Sciences Research Council and the Medical Research Council, and the University of Edinburgh as part of the cross-council Lifelong Health and Wellbeing initiative (MR/K026992/1)

Sylvia Aitken Charitable Trust

Title: Postmortem analyses of the Lothian Birth Cohort 1936: Extending brain phenotyping to the level of the synapse

Authors: *T. L. SPIRES[1], C. SMITH[2], A. WRIGHT[3], M. E. BASTIN[2], J. M. STARR[4], J. M. WARDLAW[2], T. H. GILLINGWATER[3], I. J. DEARY[4];
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Abstract: The Lothian Birth cohort of 1936 (LBC 1936) is a rare group of people for whom there are childhood cognitive test scores, longitudinal cognitive data during ageing, detailed structural MRI, genome-wide genotyping, and a multitude of other biological, psycho-social, and epidemiological data. In vivo studies of the cohort have revealed determinants of lifetime cognitive changes and cognitive changes in older age. Many studies indirectly implicate synapses as essential in the state of cognitive ageing in the human brain; however, until very recently, it was prohibitively difficult to do in-depth analyses of synaptic structure and protein composition in humans. We have applied a new method of tissue preparation at autopsy to add the study of synapses using array tomography and electron microscopy to the participants from the LBC who volunteer to donate brain tissue. Here we present data from a pilot case which details the in depth postmortem studies to be used for this cohort, including the preparation of 38 brain regions for multiple techniques (biochemistry, histopathology, EM, and array tomography).

The pilot study indicates that compared to an Alzheimer's disease patient, the cognitively normal LBC 1936 participant has a remarkable degree of preservation of synaptic structures. We do observe occasional degenerating structures (presynaptic boutons, postsynaptic dendritic spines, and synaptic mitochondria) in areas of the brain implicated in cognition (prefrontal cortex, anterior cingulate cortex, and superior temporal gyrus). This protocol for studying LBC 1936 participants postmortem extends the phenotyping of this well-characterized cohort from cognition and in vivo imaging to the level of single synapses. This will allow unprecedented studies of synaptic integrity during ageing and how it contributes to cognition.

Disclosures: T.L. Spires: None. **C. Smith:** None. **A. Wright:** None. **M.E. Bastin:** None. **J.M. Starr:** None. **J.M. Wardlaw:** None. **T.H. Gillingwater:** None. **I.J. Deary:** None.

Nanosymposium

283. Aging Brain and Cognition

Location: 152B

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 283.08

Topic: B.11. Glial Mechanisms

Support: NIH-NIMH-R01-MH085911

NIH-NIMH-R01-MH071472

Title: MFG-E8-mediated defects in microglial phagocytosis impair synaptic plasticity, learning, and memory

Authors: *N. A. DECAROLIS[1], U. HADITSCH[1], H.-R. LEE[1], M. R. SIDDIQUI[1], D. PURGER[1], K. SCHRENK-SIEMENS[1], W.-S. CHUNG[2], B. A. BARRES[2], J. B. DING[3], D. V. MADISON[4], T. D. PALMER[1];
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Abstract: Neuronal synapse elimination is an important component of plasticity, and synaptic phagocytosis by microglia contributes to activity-induced remodeling. However, the underlying mechanisms and functional consequences of altering phagocytosis in the brain remain untested. Lactadherin/MFG-E8 is required for phagocytosis of cells marked for elimination peripherally, and we hypothesized that MFG-E8 may play a role in synaptic phagocytosis in the brain. Here we show MFG-E8 is expressed in the adult brain and that microglial cells lacking MFG-E8 are

impaired in their ability to phagocytose synaptosomes in vitro. We show loss of MFG-E8 impairs hippocampus-dependent learning and memory in mice. The learning defects are accompanied by a significant decrease in synapse engulfment by microglia; an increase in the baseline number of dendritic spines; defects in long-term potentiation (LTP); alterations in basal synaptic properties; and reduction in neurogenesis. We further show that neuronal activity during learning evokes an upregulation of Mfge8 expression in the hippocampal formation in an activity-dependent manner. These data suggest that inefficient phagocytosis through loss of MFG-E8 results in the abnormal retention of inactive or defective synapses and suggest that network remodeling that is required for normal cognitive function.

Disclosures: **N.A. DeCarolis:** None. **U. Haditsch:** None. **H. Lee:** None. **M.R. Siddiqui:** None. **D. Purger:** None. **W. Chung:** None. **B.A. Barres:** None. **J.B. Ding:** None. **D.V. Madison:** None. **T.D. Palmer:** None. **K. Schrenk-Siemens:** None.

Nanosymposium

283. Aging Brain and Cognition

Location: 152B

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 283.09

Topic: B.11. Glial Mechanisms

Support: NIH Grant 1F32AG039220

S.D. Bechtel, Jr. Foundation

MetLife Foundation

Title: Astrocytic adenosine receptor A2A regulates memory

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Abstract: Adenosine is a potent neuromodulator released under normal and pathological conditions. The Gs-coupled adenosine receptor A2A is highly expressed in the brain and has

been implicated in diverse neuropathologies, including Parkinson's disease, ischemic brain injury and traumatic brain injury. These broad effects might be at least partly due to A2A receptors found in glial cells and their roles in neuroinflammatory and neuromodulatory processes. A2A receptors have also been implicated in Alzheimer's disease (AD), a neurodegenerative disorder that causes memory loss, synaptic and network dysfunctions, and alterations in glial cells. We found that astrocytes, but not microglia, had increased levels of A2A receptor expression in humans with sporadic AD and these increases correlated with disease progression. Similar to humans with AD, aging transgenic mice expressing human amyloid precursor protein (hAPP) showed increased levels of astrocytic A2A receptors and these increases correlated with the formation of amyloid plaques. We next investigated the effects of astrocytic A2A receptors and Gs-coupled signaling on learning and memory using conditional genetic ablation and chemogenetic stimulation. We found that conditional genetic removal of astrocytic A2A receptors enhanced long-term memory and Arc/Arg3.1 levels in mice without hAPP expression, suggesting that these receptors allow astrocytes to regulate memory. In support of this hypothesis, chemogenetic activation of astrocytic Gs-coupled receptor signaling reduced memory in mice without affecting learning. Conditional genetic removal of astrocytic A2A receptors enhanced memory also in aging hAPP mice without affecting their learning deficits. Additional results suggest that pathological accumulation of amyloid-beta peptides, but not tauopathy or expression of apolipoprotein E4, contributes to the increased astrocytic A2A receptor levels we observed in postmortem brain tissues from humans with AD. Together, these findings establish a regulatory role for astrocytic Gs-coupled receptors in memory and suggest that AD-linked increases in astrocytic A2A receptor levels contribute to memory loss.

Disclosures: **A.G. Orr:** None. **E.C. Hsiao:** None. **M.M. Wang:** None. **K. Ho:** None. **D.H. Kim:** None. **X. Wang:** None. **W. Guo:** None. **J. Kang:** None. **G. Yu:** None. **A. Adame:** None. **E. Masliah:** None. **B.R. Conklin:** None. **L. Mucke:** None. **N. Devidze:** None. **D. Dubal:** None.

Nanosymposium

283. Aging Brain and Cognition

Location: 152B

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 283.10

Topic: C.05. Aging

Support: NIH R01AG041944 (SLP)

Title: Aging-associated immune dysregulation drives a shift in the direction of hippocampal synaptic plasticity and increases mini frequency

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Abstract: Older individuals often experience precipitous declines in cognitive function after challenges (Ex. infection, injury, surgery) to the peripheral immune system. Aging sensitizes the hippocampal inflammatory response to peripheral infection, increasing the magnitude and duration of interleukin-1beta (IL-1β) production. We have previously demonstrated that in aging (24 month), but not in young (3 month) F344xBN rats, a peripheral immune challenge (i.p. injection of live E. coli) triggers an exaggerated elevation in hippocampal IL-1β, which in turn disrupts forms of long-term memory and synaptic plasticity known to be BDNF-dependent (Barrientos et al. 2006; Chapman et al., 2010). Hippocampal memory processes are thought to involve shifts in the balance of long-term potentiation (LTP) and depression (LTD) of excitatory synaptic transmission. Several studies have linked disruptions in hippocampal LTP, and enhancements of hippocampal LTD with memory impairments. In addition, shifts in the direction of hippocampal synaptic plasticity have been reported in rodent models of neurodegenerative disease (Li et al., 2009). Interestingly, BDNF modulates both LTP and LTD. BDNF is synthesized as a precursor protein (proBDNF), and cleaved to produce the mature BDNF protein isoform (mBDNF). Pro-BDNF binds preferentially to the pan-neurotrophin receptor p75NTR, activates apoptosis-related signaling pathways, and facilitates long-term depression (LTD) in the hippocampus. In contrast, mBDNF binds to TrkB receptors, promotes cell survival, and is required for some forms of long-term potentiation (LTP). We have previously found that mBDNF, but not pro-, is significantly reduced in hippocampal synaptoneurosomes prepared from aged animals following an infection (Cortese et al., 2011) - an observation consistent with reduced theta burst L-LTP at Schaffer collateral-CA1 synapses in these animals (Chapman et al., 2010). More recently, we have found that the IL-1β -driven shift in the ratio between proBDNF and mature BDNF is also associated with enhanced LTD. We are now more closely examining the impact age and infection on synaptic function, using whole-cell patch recording, and focusing on minis and AMPA / NMDA ratios. Interestingly, our preliminary results suggest that mini frequency is dramatically increased in aged animals with a recent history of infection. This work may provide novel insights into the early stages of synaptic failure in a variety of disorders associated with dysregulated brain inflammatory responses (Ex. post-operative cognitive dysfunction, autoimmune diseases, depression, PTSD and some neurodegenerative disorders).

Disclosures: S.L. Patterson: None. **G.P. Cortese:** None. **J.A. Varela:** None. **R.M. Barrientos:** None. **S.F. Maier:** None.

Nanosymposium

283. Aging Brain and Cognition

Location: 152B

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 283.11

Topic: C.05. Aging

Support: FCT/PTDC/SAU-NSC/099853/2009

FCT/ PTDC/SAU-NSC/122254/2010

DARPA (09-68-ESR-FP-010)

DARPA (W911NF-10-1-0059)

QREN (CENTRO-07-ST24-FEDER-002006).

EMBO short term fellowship

Investigator FCT program

Title: A novel role of adenosine A2A receptor in the modulation of the stress glucocorticoid receptor in the brain

Authors: *L. V. LOPES[1], V. L. BATALHA[1], D. R. FERREIRA[1], J. S. VALADAS[1], J. E. COELHO[1], R. GOMES[1], P. M. CANAS[2], V. BUÉE-SCHERRER[3], S. HUMEZ[3], T. SHMIDT[4], M. HAMDANE[3], G. SADRI-VAKILI[5], L. BUÉE[3], T. F. OUTEIRO[6], R. A. CUNHA[2], M. BADER[7], D. BLUM[3];
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Abstract: Here we report that adenosine A2A receptors are overexpressed in aged and Alzheimer's disease human cortex compared to young subjects. This change in receptor expression is also accompanied by down-regulation of the glucococorticoid receptor (GR). Thus, we generated transgenic rats that overexpress adenosine A2A receptors under the control of the CaMKII promoter, tg (CaMKII-hA2AR), in order to evaluate its impact on cognitive decline. Our findings demonstrate for the first time that a forebrain selective neuronal increase in A2AR

drives aging-like hippocampal deficits, such as impairments in memory tasks and HPA-axis dysfunction. Moreover, A2AR overactivation modulated glucocorticoid (GR)-induced deficits in hippocampal synaptic plasticity, increasing susceptibility to activation by the GR agonist, dexamethasone. Conversely, blockade of A2AR prevented dexamethasone-induced GR transcriptional activity and nuclear translocation. Accordingly, A2AR blockade therapy in vivo increased histone H3 acetylation of the Nr3c1 gene. Together, our results suggest that A2AR directly modulate GR, unveiling an important therapeutic alternative to GR antagonists for clinical applications. These findings are significant for the treatment of not only psychopathologies but can also be extended to the multiple age-related conditions where glucocorticoid response is impaired.

Disclosures: **L.V. Lopes:** None. **V.L. Batalha:** None. **D.R. Ferreira:** None. **J.S. Valadas:** None. **J.E. Coelho:** None. **R. Gomes:** None. **P.M. Canas:** None. **V. Buée-Scherrer:** None. **S. Humez:** None. **T. Shmidt:** None. **M. Hamdane:** None. **L. Buée:** None. **T.F. Outeiro:** None. **R.A. Cunha:** None. **M. Bader:** None. **D. Blum:** None. **G. Sadri-Vakili:** None.

Nanosymposium

283. Aging Brain and Cognition

Location: 152B

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 283.12

Topic: C.05. Aging

Support: NIA Grant R37 AG-11230

BCBSM Grant 1893.SAP

Title: Longitudinal iron accumulation is associated with regional brain shrinkage in healthy aging

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Abstract: Accumulation of non-heme iron in the brain has been hypothesized as a cellular mechanism underlying global neural decline in normal aging and neurodegenerative disease. Relatively few studies of brain iron in normal aging exist and extant studies are almost exclusively cross-sectional. Thus, the purpose of this study was to evaluate changes in brain iron

content over time, and to compare change in iron content with change in regional volume, a hallmark of aging. Iron content was estimated in vivo via T2* relaxometry, in which lower values correspond to proportionally greater iron content. T2* and volumes were measured in several brain regions in a lifespan sample of healthy adults (N = 89; age 19-77 years at baseline) that was measured twice, two years apart. Latent change score models estimated longitudinal change in regional iron content and volume, individual differences in change, and the effects of cardiovascular risk factors as modifiers of change trajectories. Iron significantly increased (T2* decreased) over time in the striatum, but neither in the globus pallidus nor in the hippocampus. The accumulation of regional iron partially accounted for shrinkage in the striatum. Significant individual differences in change in estimated iron content and volume were partially explained by sub-clinical increase in several vascular risk factors. Elevated metabolic risk indicators were associated with greater iron content at baseline, which in turn accounted for individual differences in shrinkage. Notably, baseline iron content in the striatum predicted regional shrinkage two years later. The results of this study present the first longitudinal evidence in support of iron as a biomarker of decline in healthy aging.

Disclosures: A. Daugherty: None. **N. Raz:** None.

Nanosymposium

283. Aging Brain and Cognition

Location: 152B

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 283.13

Topic: C.05. Aging

Support: Nordea Fonden for Center for Healthy Aging

NOVO-Nordisk Foundation

Lundbeck Foundation

The Danish Medical Research Council

Foundation Leducq

Title: Decreased interneuron function associates gamma rhythm to reduced oxygen supply, but increased consumption in brain aging

Authors: *S. B. JESSEN[1,2], C. MATHIESEN[1], B. L. LIND[1], M. LAURITZEN[1,3,4];
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Abstract: During aging global cerebral blood flow (CBF) is reduced, while the cerebral metabolic rate of oxygen (CMRO2) is relatively preserved in humans. The neuronal substrate for this change in function is incompletely understood. We here examined the hypothesis that an overall reduction in activity of cortical interneurons explained the decline in CBF and CMRO2. We focused on parvalbumin positive (PV) interneurons, which are fast spiking and induce neuronal network oscillations in the gamma frequency range. Gamma oscillations correlate strongly with hemodynamic responses in both humans and rodents and evoke large increases in CMRO2. Gamma oscillations are believed to underlie higher cognitive functions and disruptions in gamma rhythm and function of parvalbumin positive interneurons are associated with cognitive decline seen in CNS disorders, such as schizophrenia and Alzheimer's disease. Using 2-photon microscopy, we examined PV interneuronal Ca2+ signals in vivo in relation to CBF and CMRO2 in adult and aged mice. We report, that evoked CBF responses, synaptic activity and gamma oscillations decreased in old mice as compared to adult mice. The decline in gamma activity was consistent with a decrease in Ca2+ activity in PV perisomatic boutons, and in postsynaptic neurons receiving presynaptic perisomatic innervation in old as compared to adult mice. In comparison, the stimulation-induced rise in CMRO2 became larger; suggesting that the metabolic costs of evoked synaptic activity was increased in old animals. These results suggest that PV innervation is selectively affected by aging and that changes in function of PV interneurons may be an indicator of healthy aging. The age-dependent increase in energetic costs of synaptic activity is consistent with a decline in energetic reserve capacity with age and disrupted network deficiency leading to age-related cognitive decline.

Disclosures: S.B. Jessen: None. **C. Mathiesen:** None. **B.L. Lind:** None. **M. Lauritzen:** None.

Nanosymposium

283. Aging Brain and Cognition

Location: 152B

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 283.14

Topic: C.05. Aging

Support: Jane Coffin Childs Postdoctoral Fellowship

Stanford/NIH/National Center for Research Resources CTSA-UL1-RR025744

NIH AG045034

NIH F31 AG040877-01A1

CIRM

Anonymous

Title: Factors present in young plasma enhance neuronal function in old mice

Authors: *J. M. CASTELLANO[1], K. I. MOSHER[1], R. J. ABBEY[1], D. BERDNIK[1], J. C. SHEN[1], M. ANGST[2], T. WYSS-CORAY[1];
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Abstract: Aging is a major driving force for cognitive deficits in healthy individuals and the strongest risk factor for many neurodegenerative diseases. As the population ages, it will be critical to develop therapies that target brain aging in order to stave off the rising toll of neurodegeneration and age-associated loss of cognitive function. We and others have shown that the systemic environment may play a role in regulating how certain areas of the brain age. Exposure of old mice to young blood rejuvenates aspects of neuronal function at the molecular and cellular level, and isolated young plasma improves learning and memory when injected in old mice. We sought to understand how the plasma proteome changes with age and whether some of these changes can be exploited to uncover factors that rejuvenate processes of brain function in the aged mouse. Given that heat-denaturation of young plasma ablates its rejuvenating effects on cognition, we hypothesized that soluble factors present in young blood play a role in brain rejuvenation. Using antibody-protein arrays, we found that a large number of human plasma proteins decrease significantly with age, several of which are involved in growth and differentiation according to DAVID and Ingenuity Pathway analyses (IPA). We find that several of these novel factors increase neural progenitor cell proliferation in vitro and have strong effects in the brain when injected peripherally. We also find that treatment with different fractions of young plasma result in distinct transcriptional profiles in old mouse hippocampus, allowing us to focus on several factors as targets for restoring cognitive function in old mice. Taken together, our results suggest that many factors are responsible for rejuvenating effects of young blood on the brain, several of which may be useful in harnessing the restorative potential of young blood for the possible treatment of aging conditions.

Disclosures: **J.M. Castellano:** None. **K.I. Mosher:** None. **R.J. Abbey:** None. **D. Berdnik:** None. **J.C. Shen:** None. **M. Angst:** None. **T. Wyss-Coray:** Other; Co-founder of a company related to this work.

Nanosymposium

284. Ischemia: Cellular Mechanisms and Neuroprotection I

Location: 146C

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 284.01

Topic: C.08. Ischemia

Title: Stroke-induced vascular and astroglial changes depend on age and brain regions: Time for transfer of the 'neurovascular unit' concept into analyses of Alzheimer-like alterations?

Authors: *W. HARTIG[1], C. A. HAWKES[2], R. ANDERS[1], S. NISSEL[1], C. HOBOHM[3], J. GROSCHE[1], I. BECHMANN[4], R. O. CARARE[2], D. MICHALSKI[3];
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Abstract: Stroke research has recently switched from an initial neurocentric view of tissue damage to a broader perspective comprising the whole 'neurovascular unit' (NVU) that involves the vasculature and allocated astrocytes. However, a comparable concept for neurodegenerative pathologies like Alzheimer's disease (AD) is still under consideration. Since recent data indicate a significant impact of ischaemia and the vasculature in AD, such an approach might provide new insights into the complex AD pathophysiology. Therefore, we analysed age-dependent vascular and astroglial alterations caused by experimental focal cerebral ischaemia in a triple-transgenic (3xTg) mouse model of AD (Oddo et al. 2003, Neuron 39: 409-421) compared to wild-type (WT) mice. Three- and 12-months-old 3xTg and WT mice underwent filament-based permanent middle cerebral artery occlusion. On the next day, animals were perfused with phosphate-buffered paraformaldehyde followed by post-fixation in the same fixative. Coronal forebrain sections were applied to immunofluorescence labelling of collagen IV and laminin as typical basement membrane constituents, completed by lectin-histochemical staining with Solanum tuberosum lectin (STL) as endothelial marker. Thereby, analyses focused on neocortex, striatum and hippocampus. Subsequent immunolabelling of aquaporin-4 and CD31 detected concomitant changes of astroglial endfeet in close vicinity to the endothelium. Overall, we observed a strong up-regulation of collagen IV and laminin in neocortical areas of 3-months-old 3xTg and WT mice, whereas STL-staining appeared nearly unaffected. Quantification of collagen IV-immunoreactivity revealed up-regulation in the ischaemic neocortex of 3- and 12-month-old WT and 3xTg mice, while striatal alterations were limited to young WT mice. Unexpectedly, collagen IV expression remained unaffected in the hippocampus as an area known to be sensitive to ischaemia. Data on aquaporin-4 and CD31 immunolabelling indicated more severe astrocytic and endothelial degeneration in 3xTg mice (see also Hawkes et al. 2013, Exp

Neurol 250: 270-281). In conclusion, these findings indicate a central role of the cerebral vasculature and associated astroglial endfeet during the pathogenesis of AD. Consequently, the consideration of the NVU concept in future studies of AD is strongly recommended.

Disclosures: **W. Hartig:** None. **C.A. Hawkes:** None. **R. Anders:** None. **S. Nissel:** None. **C. Hobohm:** None. **J. Grosche:** None. **I. Bechmann:** None. **R.O. Carare:** None. **D. Michalski:** None.

Nanosymposium

284. Ischemia: Cellular Mechanisms and Neuroprotection I

Location: 146C

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 284.02

Topic: C.08. Ischemia

Title: Roles of pericyte NADPH oxidase 4 in acute brain ischemia

Authors: *A. NISHIMURA[1,2], T. AGO[2], Y. YOSHIKAWA[2], M. TACHIBANA[2], R. MATSUO[2], Y. WAKISAKA[2], J. KURODA[2], T. KITAZONO[2];
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Abstract: Pericytes, mural cells in capillary vessels, exist abundantly in the brain and compose the neurovascular unit with endothelial cells, glial cells, and neurons. It has been elucidated that pericytes play a key role in the formation and maintenance of the blood-brain barrier (BBB) because pericytes crucially affect the expression of proteins related to tight junction formed by endothelial cells and produce matrix metalloproteinases (MMPs) that regulate BBB stability. The NADPH oxidase family proteins are major reactive oxygen species (ROS)-producing enzymes. We have reported that Nox4 is abundantly expressed in pericytes. Our goal was to elucidate the roles of Nox4 in brain pericytes during acute brain ischemia. We confirmed by quantitative PCR (qPCR) that Nox4 was expressed in human cultured brain microvascular pericytes (HBMPC) among the NADPH oxidase family and was significantly upregulated by hypoxia (at 1% O2 for 48 hours) by 7.8-fold ($p < 0.05$). We produced a mouse MCAO stroke model and examined the expression pattern of Nox4 in the brain. Immunofluorescent double labeling demonstrated that Nox4 expression was upregulated in microvessels particularly in peri-infarct areas and was co-stained with PDGFRβ, a pericyte marker. In order to elucidate the role of Nox4 in brain pericyte during brain ischemia, we generated mice with pericyte-specific human Nox4 overexpression using an SM22α promoter (Tg-Nox4). We confirmed that SM22α was expressed both in HBMPC by immunoblot and in mouse brain pericytes by co-immunostaining with PDGFRβ. We

isolated microvessels from Tg-Nox4 brain and confirmed that human Nox4 mRNA was highly expressed in the vessels. We applied Tg-Nox4 to a MCAO model, and found that infarct volume was significantly larger in Tg-Nox4 than in littermate controls. Confocal laser scanning microscopy demonstrated that IgG leakage, an indicator of BBB breakdown, in peri-infarct areas was significantly increased in Tg-Nox4, suggesting that Nox4 overexpression in pericytes enhanced BBB breakdown during brain ischemia. To elucidate the mechanisms underlying the Nox4-mediated increased BBB breakdown, we induced adenoviral-mediated overexpression of Nox4 in HBMPC. We demonstrated that Nox4 overexpression increased NFκB phosphorylation and MMP9 expression in the cells. We also comfirmed that NFκB phosphorylation and MMP9 activity was increased in Tg-Nox4 mouse. In conclusion, Nox4 may be a major source of ROS in brain pericytes and is upregulated directly by hypoxia in peri-infarct areas during acute brain ischemia. Pericyte Nox4 may enhance BBB breakdown through the activation of NFκB-MMP9 signaling during acute brain ischemia.

Disclosures: **A. Nishimura:** None. **T. Ago:** None. **Y. Yoshikawa:** None. **M. Tachibana:** None. **R. Matsuo:** None. **Y. Wakisaka:** None. **J. Kuroda:** None. **T. Kitazono:** None.

Nanosymposium

284. Ischemia: Cellular Mechanisms and Neuroprotection I

Location: 146C

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 284.03

Topic: C.08. Ischemia

Support: NIH Grant EY004077

Fondation Leducq

NIH T-32 GM 008244

American Heart Association Predoctoral Fellowship

Title: Glial calcium signaling in the ischemic mouse retina

Authors: ***A. I. SRIENC**[1], K. BIESECKER[1], A. AGARWAL[2], D. E. BERGLES[2], E. A. NEWMAN[1];
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Abstract: Under pathologic conditions in the brain, basal calcium levels and transient calcium signaling increase in glial cells. It remains unknown how glial calcium signaling changes with retinal pathology. The functional consequences of altered retinal glial calcium signaling also remain unknown. We used a photothrombosis model of retinal vessel occlusion in transgenic mice selectively expressing the genetically encoded calcium indicator GCaMP3 in glial cells. Photothrombosis-induced glial calcium signaling was monitored in the in vivo retina with confocal microscopy. Following imaging, retinas were TUNEL stained to quantify cell death. Glial calcium signaling was characterized with regard to proximity to the injury and the optic disc, and timing relative to onset of injury. Glial calcium waves were observed with increased frequency during and after photothrombosis. These calcium waves were not limited to vascular areas targeted for photothrombosis; rather, they occurred over the full surface of the imaged retina. Preliminary analysis shows that elevated glial calcium signaling is associated with elevated apoptosis. Future studies will be carried out on IP3R2 KO animals to determine whether pathologic retinal calcium signaling is IP3 dependent. Since retinal ischemia continues to be a common cause of blindness, understanding the role of retinal glial cells in ischemic damage could aid in identifying valuable therapeutic targets for preventing ischemia-induced vision loss.

Disclosures: A.I. Srienc: None. **K. Biesecker:** None. **A. Agarwal:** None. **D.E. Bergles:** None. **E.A. Newman:** None.

Nanosymposium

284. Ischemia: Cellular Mechanisms and Neuroprotection I

Location: 146C

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 284.04

Topic: C.08. Ischemia

Support: RO1NS07653=90050162

Title: Genetic engineering of Primary Glial Restricted Progenitors for improved intraarterial targeting to the ischemic brain

Authors: *A. M. JABLONSKA[1,2], D. J. SHEA[3], A. ARNOLD[1,2], J. W. BULTE[1,2], M. JANOWSKI[1,2,4,5], K. KONSTANTOPOULOS[3], P. WALCZAK[1,2,6];
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Abstract: Stroke is a leading cause of death and chronic disability in adults and to date it is lacking effective therapy. Stem cells transplantation is an attractive strategy to repair damage following cerebral ischemia. Reported preservation of axons with degradation of myelin in rodent models of subcortical stroke provides rationale for treatment with oligodendrocyte precursors. Our earlier work indicates positive effect of myelinating glial restricted progenitor (GRPs) transplantation in rat model of transverse myelitis and dysmyelinated mice. However, clinical translation is contingent upon methodology facilitating efficient targeting and broad cell distribution throughout the lesion. Local injections, effective in small animals, failed in several clinical trials. Intraarterial route is a strategy that may result in efficient cell delivery to stroke lesion; however, it requires passage of cells across the capillary. Studies with immortalized GRPs showed that overexpression of VLA4, adhesion molecule results in their capture on activated brain endothelium in rats. Though immortalized cells are relatively easy to transfect they are unsuitable for clinical use. Aim of this study is to transiently overexpress VLA4 in therapeutic, primary GRPs and assess functionality of the transgene in vitro and in vivo. Transfection efficiency with DNA plasmids for integrin α4 and β1 optimized for the primary GRPs reached over 60%. Functionality of the transgene was tested in microfluidic adhesion assays. Perfusion of VLA4+GRPs through microfluidic channels coated with VCAM1 protein showed their slowed rolling compared to naïve GRPs. Adhesion experiment with human brain endothelial cells coated channels revealed higher number of VLA4+GRPs binding to endothelial cells activated with TNFα. With these data we initiated animal studies with assessing endothelial capture and diapedesis in rodent model of stroke using intravital multi-photon microscopy. Using a method for visualization of brain vasculature based on systemic injection of TxRed, Dextran® 70000MW (4.5mg/300µl; Life Technologies) we demonstrated that intraarterially injected VLA4+GRPs effectively bind to endothelial cells. Ongoing studies are focusing on assessing their extravasation to brain parenchyma. In summary, we demonstrated that overexpression of VLA4 in primary GRPs results in their improved adhesion to brain endothelium both in vitro and in vivo.

Disclosures: **A.M. Jablonska:** None. **D.J. Shea:** None. **A. Arnold:** None. **J.W. Bulte:** None. **M. Janowski:** None. **K. Konstantopoulos:** None. **P. Walczak:** None.

Nanosymposium

284. Ischemia: Cellular Mechanisms and Neuroprotection I

Location: 146C

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 284.05

Topic: C.08. Ischemia

Support: KAKENHI (26860851)

KAKENHI (23700471)

Title: Cell death and arrest of lineage progression to oligodendrocyte are followed by indirect damage of corticospinal neurons in the developing white matter injury model rat

Authors: ***S. MISUMI**, Y. UEDA, Y. SHIMIZU, A. ISHIDA, C.-G. JUNG, H. HIDA; Nagoya City Univ. Grad Sch., Nagoya, Japan

Abstract: Developing white matter injury (DWMI) caused by perinatal hypoxia-ischemia (H-I) is associated with permanent neurodevelopmental disabilities in preterm infants. We previously showed a DWMI model rat that was made by H-I (right common carotid artery occlusion followed 6% hypoxia for 1 hour) at P3. We first characterized motor functional deficits of DWMI model: several behavioral tests (rotarod, grip test, horrizontal ladder test, and gait analysis) were performed. No significant difference in grip test and horizontal ladder test was found between DWMI and control (hypoxia only). Less score in Rota rod and different paw angle of hindlimb in gait analysis was observed in DWMI, revealing that hindlimb function was mainly disturbed in this model. We next investigated the pattern of cell damage in sensorimotor cortex after H-I. Most of active caspase-3-positive cells were oligo-2-positive cells in sensorimotor cortex 24 hours later, while apoptotic NeuN-positive neurons were not detected. NG-positive cells increased in the ipsilateral white matter at 2 day and PDGFRα -positive cells also increased at 7 day after H-I, indicating oligodendrocyte progenitors (OPC) transiently increased in DWMI model. However, MBP was weakly stained in hindlimb area 2 months later. We further investigated whether indirect damage in the corticospinal neurons was induced after H-I. Retrograde tracer into the corticospinal neurons at P20 showed a tendency to reduction of the neurons in motor area in DWMI model (77.5 ±3.34 % of control, p=0.09). Data suggest that cell death of OPC and arrest of the cell lineage progression were induced in DWMI model followed by secondary minor damage of the corticospinal neurons, relating to impaired hindlimb motor function.

Disclosures: **S. Misumi:** None. **Y. Ueda:** None. **Y. Shimizu:** None. **A. Ishida:** None. **C. Jung:** None. **H. Hida:** None.

Nanosymposium

284. Ischemia: Cellular Mechanisms and Neuroprotection I

Location: 146C

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 284.06

Topic: C.08. Ischemia

Support: KAKENHI 23700471

KAKENHI 26860851

Title: Response to intracortical microstimulation in the hindlimb area in the developmental white matter injury model rat

Authors: *Y. UEDA, S. MISUMI, A. ISHIDA, Y. SHIMIZU, C.-G. JUNG, H. HIDA; Neurophysiol. & Brain Sci., Nagoya City Univ. Grad. Sch. Med. Sci., Nagoya, Japan

Abstract: Developing white matter injury (DWMI) caused by perinatal hypoxia-ischemia (H-I) is associated with permanent neurodevelopmental disabilities such as motor and cognitive dysfunction in preterm infants. A DWMI model was made by right common carotid artery occlusion followed by 6% oxygen for 1h using P3 Wistar rat. We showed that impaired motor function especially in the hindlimb was observed in our DWMI model. However, whether electrical responsiveness in the cerebral cortex of hindlimb area is altered by H-I remains unclear. To investigate the response to electrical stimulation in the sensorimotor cortex, intracortical microstimulation (ICMS) was carried out in adult DWMI model rat. Under anesthesia (ketamine and xylazine), following to gentle craniotomy, bipolar pulses (0.2 ms, 0-200 μA, 333 Hz) were given to tungsten electrode in layer V sensorimotor cortex (AP: 1.0~3.0 mm from the bregma, ML: 1.0~3.0 mm), detecting the evoked twitches in contralateral side. We first checked the body portion moved and then measured the threshold of the current that can evoke the twitch. It found that the largest field of the response map was hip joint in sham group rats. It is revealed that the area of hip joint became smaller in DWMI model rat compared to control, while the area of trunk became bigger. However, no significant difference of the threshold was found between DWMI model rat and control. Data suggest that cortical map of hindlimb area is significantly altered in DWMI model rat without changing the threshold, probably related to impaired hindlimb motor function.

Disclosures: Y. Ueda: None. **S. Misumi:** None. **A. Ishida:** None. **Y. Shimizu:** None. **C. Jung:** None. **H. Hida:** None.

Nanosymposium

284. Ischemia: Cellular Mechanisms and Neuroprotection I

Location: 146C

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 284.07

Topic: C.08. Ischemia

Support: The Sheila S. and Lawrence C. Pakula, M.D. Endowment for Neonatal Research

Title: Ontogenic and sexual differences in neurotrophin response to anesthesia and neonatal HI in cerebellum

Authors: *J. YU, D. L. FLOCK, F. J. NORTHINGTON, R. CHAVEZ-VALDEZ;
Johns Hopkins, Baltimore, MD

Abstract: Background: Advances in imaging techniques are improving the identification of cerebellar injuries linked to prematurity and/or hypoxia-ischemia (HI). Although 25-30% of premature infants have cerebellar neuronal loss and 90-100% of them have gliosis at baseline (Volpe 2009), the effects of anesthesia and HI in the cerebellum of this vulnerable population remain unknown. Furthermore, the ontogenic changes in the susceptibility to cerebellar injury are also unknown. Since neurotrophins, such as BDNF and NT4 promote: i) survival, axonal elongation and dendritic arborization of Purkinje cells (Gao 1995; Schwartz 1997), and ii) their exogenous treatment extends cerebellar climbing fiber development (Sherrard 2001), we aim to study the effects of anesthesia and HI in BDNF and NT4. We hypothesize that anesthesia and HI modulate the cerebellar neurotrophic profile and that this modulation will vary by gender and developmental maturity. Method: At postnatal day (p)7 (preterm) and p10 (term), C57B6 mice were subjected to: i) nothing (naive); ii) anesthesia with isoflurane (sham), or iii) HI via unilateral carotid ligation followed by hypoxia (45 min, 8% O2). 24h after procedure pups were euthanized and cerebellum collected (n=5/group). BDNF and NT4 gene expression were measured by qRT-PCR. Result: In the p7 model at 24h, male mice BDNF mRNA levels in cerebellum were 40% lower in sham (p=0.013 vs. naive), while 40% (p=0.033 vs. naive) and 100% (p=0.003 vs. sham) higher in HI. In female mice BDNF levels were unchanged with anesthesia or HI. Similarly, NT4 remained unchanged in both sexes regardless of treatment. In the p10 model at 24h, while neither treatment changed BDNF levels in male mice, HI increased BDNF by 2.8 (p=0.015 vs. naive) and by 2.5 (p=0.008 vs. sham) in female mice. NT4 levels trend to decrease 60% in male sham (p=0.06 vs. naive), while HI produced no changes. In female mice, NT4 levels decreased 60% in sham (p=0.03 vs. naive) similar to males, while NT4 increased 60% in HI (p=0.021 vs. sham). Conclusion: This is the first study describing neurotrophin gene expression in the neonatal mouse cerebellum and alterations following HI and anesthesia. While BNDF mRNA expression decreases with anesthesia exposure in males in a preterm model, this effect is not seen in the term model. Instead decreases in NT4 in both sexes characterize anesthesia exposure in the term model. On the other hand, HI is linked to increase i) BDNF expression in preterm males and in term females, and ii) NT4 expression in term females.

Therefore, neurotrophic responses to anesthesia and HI differ by sex and development in the cerebellum suggesting differences in susceptibility to cerebellar injury.

Disclosures: J. Yu: None. **D.L. Flock:** None. **F.J. Northington:** None. **R. Chavez-Valdez:** None.

Nanosymposium

284. Ischemia: Cellular Mechanisms and Neuroprotection I

Location: 146C

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 284.08

Topic: C.08. Ischemia

Support: CNPq

FAPERJ

SR-2 UERJ

Title: Developmental effects of prenatal hypoxia-ischemia: Glutamate receptors and transporters and cell communication *in vitro*

Authors: *M. C. RODRIGUES[1], C. T. N. BALDUCI[1], A. P. COSTA[1], T. SAVIGNON[2], F. TENÓRIO[1], C. HEDIN-PEREIRA[3], P. C. BARRADAS[1];
[1]DFP, State Univ. of Rio De Janeiro, Rio De Janeiro, Brazil; [2]Oswaldo Cruz Fndn., Rio De Janeiro, Brazil; [3]Federal Univ. of Rio de Janeiro, Rio De Janeiro, Brazil

Abstract: Hypoxic-ischemic (HI) infant human show oligodendrocyte loss, astrogliosis, cortical development and motor behavior impairments including cerebral palsy. Cerebellum plays a role in motor control and many damages have been demonstrated in HI humans and animals. Glutamatergic excitotoxicity is usually associated to HI and cellular junctions may be able to modulate HI effects. Previous data from our group using a model of prenatal HI in rats have shown long-lasting damages in cerebellar structure and indicate that deleterious effects may be sustained until adult life. Our objectives were to characterize connexin (Cx) and glutamate receptors and transporters levels during the development of HI cerebellum and to assess gap junctions in cerebellar astrocyte cultures derived from rats submitted to the same model. Anesthetized rats on the 18th gestation day had the 4 uterine arteries clamped for 45 minutes (HI group). Control animals had the uterine horns exposed but no arteries were clamped (SH group). Project approved by University Ethics Committee (CEA-UERJ 019/2010). Cerebella of pups at 2

(P2), 9 (P9), 16 (P16), 23 (P23), 30 (P30), 45 (P45) and 90 (P90) postnatal days were submitted to Western blot using anti-NR2B, anti-GluR3, anti-EAAT1, anti-GFAP and anti-Cx43 antibodies (n=3-7). P2 cerebella were used in astrocyte primary cultures which were immunostained with anti-Cx43, anti-GFAP, anti-nestin and anti-A2B5 antibodies (n=5). Our results show differences in GluR3 levels along development of SH and HI animals, with a significant decrease of this subunit in HI group at P9 (SH:7,394±0,776; HI:4,504±0,560; $p<0,05$). There is also an increase of EAAT1 levels in HI animals when compared to SH at P90 (SH:22,064±3,989; HI:42,568±4,681; $p<0,05$). However we did not observe any variation in NR2B and GFAP levels between groups at different ages. We also verified significant decreased Cx43 levels in HI group at P2 (SH:18,328±0,096; HI:16,004±0,115; $p<0,05$) and in cultured astrocytes which also had morphological modifications and different patterns of A2B5 marker expression. The modification related to GluR3 in HI may be caused by impaired dendritic arborization or by a reduced number of oligodendrocyte progenitors at P9, already described by our group. Cx43 reduction indicates that substance traffic may be impaired and contribute to lesion expansion. Astrocyte differentiation changes may reflect potential effects of HI on long-term cell maturation. Our results confirm that prenatal HI may be responsible for changes that characterize glutamatergic excitotoxicity. We reassure the importance of astrocyte communication as a neuroprotective strategy in this lesion.

Disclosures: **M.C. Rodrigues:** None. **C.T.N. Balduci:** None. **A.P. Costa:** None. **T. Savignon:** None. **F. Tenório:** None. **C. Hedin-Pereira:** None. **P.C. Barradas:** None.

Nanosymposium

284. Ischemia: Cellular Mechanisms and Neuroprotection I

Location: 146C

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 284.09

Topic: C.08. Ischemia

Support: Basque Country Government (IT773/13)

Fundación Jesus de Gangoiti Barrera

Title: Study of the auditory threshold in a perinatal asphyxia model. A model to study auditory system impairment

Authors: A. MARTINEZ-IBARGÜEN[1], M. REVUELTA[2], O. ARTEAGA[2], H. MONTALVO[2], H. LAFUENTE[4], F. ALVAREZ[5], D. ALONSO-ALCONADA[2], *L.

MARTINEZ MILLAN[3], E. HILARIO[2], A. ALVAREZ[2];
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Abstract: Hearing is a fundamental sense for communication skills mostly acquired in the first years of life. The normal development and myelination of the auditory system can be impaired by certain pathological conditions such as viral infections, meningitis, fetal distress and perinatal asphyxia. To evaluate the auditory capacity in newborns, it is recommended the use of quantitative measures methods such as auditory evocated potentials (AEPs) that reflect the voltage variation produced during the auditory pathway after a short acoustic stimuli. The aim of the present work was to determine the functional integrity of the auditory pathway by the measure of the AEPs using a model of hypoxic-ischemic brain injury in newborn piglets. Hypoxia-ischemia was induced to 1.3 day-old piglets by clamping both carotid arteries by using vascular occluders and lowering the fraction of inspired oxygen to 8-10% over 20 min . We compared the Auditory Brain responses (ABRs) of newborn piglets exposed to acute hypoxia/ischemia (n=6) and a control group with no such exposure (n=10) by GSI Audera equipment. Repeated ABRs were recorded for both ears of all of the animals under baseline conditions, during the stabilization phase of the animal, 20 minutes after the end of HI injury, and every 30 minutes over 6 h after the HI injury. We distinguish five waves in the first 10 ms after the stimuli generated in the anatomical structures of the auditory pathway as assessed by ABR. This Auditory Pathway was altered during the hypoxic-ischemic insult, with the disappearance of all of the responses, but it was recovered 30-60 minutes after the damage event. Hypoxia/ischemia seemed to induce auditory functional damage in the neural integrity of the brainstem by increasing I-V latencies and decreasing wave I, III and V amplitudes. The experimental model of hypoxia-ischemia in newborn piglets described is useful for studying the effect of perinatal asphyxia in the auditory system and in future treatment strategies for the hearing impairment.

Disclosures: A. Martinez-Ibargüen: None. **M. Revuelta:** None. **O. Arteaga:** None. **H. Montalvo:** None. **H. Lafuente:** None. **F. Alvarez:** None. **D. Alonso-Alconada:** None. **L. Martinez Millan:** None. **E. Hilario:** None. **A. Alvarez:** None.

Nanosymposium

284. Ischemia: Cellular Mechanisms and Neuroprotection I

Location: 146C

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 284.10

Topic: C.08. Ischemia

Support: CNPq

FAPERJ

SR2-UERJ

Title: Prenatal hypoxic-ischemic insult leads to astrogliosis in periaqueductal gray and enhances the nociception and anxiety-like behavior in adult male rats

Authors: *L. SPIEGEL DE ALMEIDA, P. C. ARAUJO, O. M. M. S. DE ALMEIDA, P. C. BARRADAS;
UERJ, Rio De Janeiro, Brazil

Abstract: Infant human brains show hypomyelination, astrogliosis, cortical development and motor behavior impairments after perinatal hypoxia-ischemia (HI) insults. It has been previously shown, in a model of HI in which the uterine arteries of pregnant rats were clamped, astrogliosis, oligodendroglial death, axonal rupture and motor deficits in the offspring. Astrocytes are involved in multiple functions including pain signaling modulation. Periaqueductal gray (PAG) is related to the descending modulation of pain and also in the modulation of aversive behavior. As chronic pain is a common complaint among the adult patients that suffered HI insults, in this work we aimed to verify the pain sensibility, anxiety levels and astrocyte responses in adult rats submitted to a model of systemic perinatal HI. Rats on the 18th gestation day were anesthetized, the uterine horns were exposed and the four uterine arteries were clamped for 45 minutes. SHAM controls had the uterine horns exposed, but no arteries were clamped. Gestation proceeded after surgery and only full term animals were used. Postnatal day 90 animals (P90) from both groups were submitted to behavioral tests (n: SH=8; HI=5). P90 animals were also submitted to histological procedure (n: SH=3; HI=3) and their brains immunoreacted with anti-GFAP antibody. In the Open Field Test (OPT) we measured the quantity of squares in center and periphery walked during 5 minutes and calculated the locomotion index, which is the number of squares of center/periphery x 100. In the Hot Plate Test (HPT), the animals stood on a hot surface (48°C) during 1 minute and we measured the time until licking the hindpaw. In the OPT, HI animals (0.0610±0.0095) presented reduction in the locomotion index ($p<0.05$) when compared with SH animals (0.0924±0.0305); in the HPT the HI animals (8.8± 1.9235) also presented reduction in withdraw latency of hindpaw ($p<0.05$) when compared with SH animals (16.875±5.9387). We observed an increase in GFAP immunostaining in the dorsomedial (SH - 57608±17188; HI - 75745± 23623; $p<0.01$), dorsolateral (SH - 54331± 14627; HI - 70536±21547; $p<0.001$), lateral (SH - 60146±16313; HI - 74725±18381; $p<0.001$) and ventrolateral (SH - 50975±9652; HI - 74841±10116; $p<0.001$) PAG in HI animals, which suggests that astrogliosis is sustained until adulthood. Our results suggest that our model leads to

the increase of nociception and anxiety levels in HI animals, which may be related to astrogliosis in PAG.

Disclosures: **L. Spiegel De Almeida:** None. **P.C. Araujo:** Other; CNPq, UERJ. **O.M.M.S. de Almeida:** A. Employment/Salary (full or part-time):; UERJ. **P.C. Barradas:** A. Employment/Salary (full or part-time):; UERJ.

Nanosymposium

286. Spinal Cord Injury: Therapeutic Strategies

Location: 152A

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 286.01

Topic: C.10. Trauma

Support: Natural medicine and biotechnology research

Title: Improvement of motor dysfunction in acute and chronic phases in spinal cord injury mice by a crude drug-derived compound

Authors: ***N. TANABE**[1], T. KUBOYAMA[1], K. KAZUMA[2], K. KONNO[2], C. TOHDA[1]; [1]Div. of Neuromedical Science, Inst. of Natural Med., Univ. of Toyama, Toyama, Japan; [2]Div. of Kampo-Pharmaceutics, Inst. of Natural Med., Univ. of Toyama, Toyama, Japan

Abstract: Several compounds were already reported to induce functional recovery when treated immediately after spinal cord injury (SCI) in mice. But few compounds were effective for SCI mice in the chronic phase. Since chondroitin sulfate proteoglycan (CSPG) deposits and inhibits axonal extension in the injured region of the spinal cord, broken neuronal networks are hardly reconstructed, and motor function is not persistently restored. Therefore, axonal extension in the inhibitory environment is one of essential events for recovery from motor dysfunction. Our previous study showed that water extract of crude drug X (X extract) enhanced axonal extension in cultured cortical neurons and improved hindlimb function and axonal growth in the acute phase in SCI mice. In the present study, we aimed to identify active constituents in X extract and investigate effects of the active constituents on motor dysfunction in acute and chronic phases in SCI mice. Axonal extension activities of compounds in X extract were evaluated in cultured cortical neurons (ddY mice, E14) on the inhibitory CPSG substrate. Four days after the treatment, axonal lengths were quantified by immunostaining for phosphorylated neurofilament-H. Although axonal extension was inhibited on the CSPG, compound A (10 μM) strongly induced axonal extension in the presence of the inhibitory CSPG. Next, an in *vivo* effect of

compound A was investigated in acute phase in SCI mice (ddY, female, 8 weeks old) suffered from contusion injury. Consecutive oral administrations of compound A (100 μmol/kg/day, for 30 days) or vehicle solution to SCI mice was started from 1h after the injury. Compound A significantly recovered motor function of hindlimbs. Additionally, an effect of compound A on SCI mice in the chronic phase was also investigated. Consecutive oral administration of compound A (100 μmol/kg/day) was started from 30 days after the injury. Administration of compound A for 3 months significantly recovered motor function of hindlimbs. In this study, we demonstrated beneficial effects of compound A on the chronic as well as the acute phase in SCI mice. Compound A is a new candidate for SCI treatment, which may be applicable to the chronic phase. *Crude drug X and compound A are not open due to patent matters.

Disclosures: **N. Tanabe:** None. **T. Kuboyama:** None. **C. Tohda:** None. **K. Kazuma:** None. **K. Konno:** None.

Nanosymposium

286. Spinal Cord Injury: Therapeutic Strategies

Location: 152A

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 286.02

Topic: C.10. Trauma

Support: CIHR

CPA

MICH

Title: Neuregulin-1 therapy moderates reactive astrogliosis and scar formation following spinal cord injury

Authors: A. ALIZADEH, S. DYCK, D. NGUYEN, S. KALLIVALAPPIL, E. PROULX, E. EFTEKHARPOUR, *S. KARIMI-ABDOLREZAEE;
Physiol., Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: Introduction: Reactive astrogliosis is a key pathophysiological event after spinal cord injury (SCI). Activated astrocytes secrete a myriad of pro-inflammatory cytokines, nitric oxide (NO) and inhibitory extracellular matrix components including chondroitin sulphate proteoglycans (CSPGs) that cause neurotoxicity and impede tissue repair and regeneration. Our recent evidence suggests that drastic downregulation of Nrg-1 after SCI may underlie astrocytes

reactivity following injury. Here, using complementary in vitro and in vivo approaches, we examined the impact of Nrg-1 on astrocyte activation. We demonstrate that availability of Nrg-1 mitigates multiple detrimental consequences of reactive astrogliosis in SCI. Methods: We utilized an in vivo rat model of incomplete compressive SCI and two in vitro models of reactive astrogliosis. In SCI rats, recombinant human Nrg-1β1 (rhNrg-1β1) was delivered intrathecally after SCI using mini-osmotic pumps and spinal cord tissue was analyzed by western blotting, immunohistochemistry and stereological techniques at different intervals post-SCI. For in vitro astrogliosis, primary rat astrocyte cultures were activated using lipopolysaccharide (LPS) or transforming growth factor-beta (TGF-β) and then Nrg-1 with or without its neutralizing antibody was added to the cultures. Astrocyte conditioned media (ACM) and cell lysate were collected and analyzed using immunocytochemistry, enzymatic assays, and Western and slot blotting to assess cellular and molecular characteristics of astrocyte reactivity including oxidative stress markers, CSPGs and proinflammatory cytokines. Results: We report that Nrg-1 treatment significantly attenuates several inhibitory and toxic aspects of reactive astrogliosis including CSPG production and the release of proinflammatory cytokines (TNF-α and IL-1β) in ACM of LPS-activated astrocytes. Additionally, Nrg-1 activation can mitigate oxidative stress in activated astrocytes by reducing their NO production. Moreover, Nrg-1 availability attenuated cell proliferation and nestin upregulation, two cellular characteristics of reactive astrogliosis. In vivo administration of Nrg-1 following SCI reduced CSPG production and chronic scar formation. The effects of Nrg-1 were specific as inactivated Nrg-1 using its neutralizing antibody failed to exhibit such effects when used in vitro. Conclusion: Our work provides novel insights into the mechanisms of astrogliosis following an injury and suggests a positive role for Nrg-1 in ameliorating the outcomes of SCI.

Disclosures: **A. Alizadeh:** None. **S. Dyck:** None. **D. Nguyen:** None. **S. Kallivalappil:** None. **E. Proulx:** None. **E. Eftekharpour:** None. **S. Karimi-Abdolrezaee:** None.

Nanosymposium

286. Spinal Cord Injury: Therapeutic Strategies

Location: 152A

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 286.03

Topic: C.10. Trauma

Support: CAPES

CNPq

FAPERJ

Title: Systemic administration of mesenchymal stem cells promotes tissue preservation and functional recovery after compressive spinal cord injury in mice

Authors: B. S. RAMALHO[1], C. M. SALES[1], A. B. MARTINEZ[1], *F. ALMEIDA[2];
[1]Univ. Federal do Rio de Janeiro, Rio de Janeiro, Brazil; [2]Histologia, UFRJ, Rio De Janeiro, Brazil

Abstract: Spinal cord injury (SCI) causes motor and sensory deficits that impair functional performance, and significantly impacts expectancy and quality of life. These functional deficits occur because of axonal degeneration, neuron and glial cells death and demyelination. The aim of this study was to investigate the effect of the systemic transplantation of mesenchymal stem cell (MSC) as a treatment in a compressive spinal cord injury model. For this purpose, we used adult female C57BL/6 mice that underwent laminectomy at T9 level, followed by spinal cord compression for 1 minute with a 30g vascular clip. One week after SCI, characterizing a subacute lesion, the animals received an intraperitoneal (i.p.) or an intravenous (i.v.) MSC injection (8 x105 in a volume of 500 μL) or vehicle (DMEM - 500 μL) as treatment. After transplantation, up to 8 weeks, we performed behavior testing using global mobility test and Basso Mouse Scale (BMS). After that, the animals were sacrificed and the samples were processed for light microscopy and immunohistochemistry. The results of cell transplanted groups revealed an improvement on locomotor performance, including a better global mobility and higher scores in BMS test in both, i.p. and i.v. groups. These animals also presented better white matter preservation in comparison to DMEM group and the semithin analysis revealed several preserved nerve fibers, and these fibers presented higher caliber and higher axonal and myelin areas. In addition, in the treated groups it was found higher levels of trophic factors and reduction in astrocytosis. So, our results suggest that the therapies used in this work showed beneficial effects, indicating that this treatment increased white matter sparing, nervous fibers preservation and contributed to functional recovery. In addition, we can also conclude that systemic transplantation of mesenchymal stem cell is a feasible choice for SCI treatment.

Disclosures: B.S. Ramalho: None. **C.M. Sales:** None. **A.B. Martinez:** None. **F. Almeida:** None.

Nanosymposium

286. Spinal Cord Injury: Therapeutic Strategies

Location: 152A

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 286.04

Topic: C.10. Trauma

Support: NIH Grant R21NS084379

Title: Local delivery of minocycline for spinal cord repair

Authors: Z. WANG[1], T. KIM[3], K. WOFFORD[1], Z. ZHANG[1], *Y. ZHONG[2];
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Abstract: Traumatic spinal cord injury (SCI) causes partial or complete functional loss below the injury site. Following the initial trauma, the lesion site expands over time due to a wave of secondary injury, which can lead to a cavity many times bigger than the initial lesion and may extend several segments above and below the site of injury. Many mechanisms and molecules contribute to the secondary injury. However, most treatment strategies are highly specific, targeting only one or a few elements in the injury cascades, and have been largely unsuccessful in clinical trials. Minocycline (MH) is a clinically available antibiotic and anti-inflammatory drug that also exhibits neuroprotective activities. It has been shown to target a broad range of secondary injury mechanisms, and protect neural tissue from multiple neurotoxic insults after SCI, via its anti-inflammatory, anti-oxidant, and anti-apoptotic properties. Systemic administration of high doses of MH has been shown to reduce secondary injury and improve functional recovery in various animal models of SCI. However, the doses required (45-90 mg/kg) in these studies are much higher than the standard human dose (3mg/kg). Even at the high dosage level of 50 mg/kg, MH level in CSF (0.5 μg/ml) is far below the level necessary for neuroprotection (1.5 – 75 g/ml in a dose-dependent manner). We have developed novel polysaccharide-MH particles self-assembled by metal ion binding for controlled and sustained delivery of MH. Injectable agarose hydrogel is used for particle encapsulation. The objective of this study is to investigate whether intrathecal delivery of high concentrations of MH for extended period of time can effectively reduce secondary injury and promote functional recovery. Adult rats were unilaterally contused at C5. Agarose hydrogel loaded with MH particles was injected into the intrathecal space at the injury site. HPLC analysis demonstrated that MH concentration was 18.8 and 12.5 μg/ml in C5 segment at day 1 and day 3 respectively. Moreover, high concentrations of MH were also detected two segments rostral and caudal to C5. Histological analysis was performed 7 days after injury. Lesion volume was significantly reduced ($P < 0.001$) by 83.7% in the MH-treated group compared with untreated control. The length of injury (3.3 mm) was statistically significantly shorter in the MH-treated group than control group (6.4 mm). GFAP and ED1 staining shows that the overall astrocyte and microglia reactivity was significantly reduced. These results suggest that local delivery of high concentrations of MH exerted potent neuroprotective effect and inhibited the spreading of secondary injury after SCI.

Disclosures: **Z. Wang:** None. **T. Kim:** None. **Y. Zhong:** None. **K. Wofford:** None. **Z. Zhang:** None.

Nanosymposium

286. Spinal Cord Injury: Therapeutic Strategies

Location: 152A

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 286.05

Topic: C.10. Trauma

Support: NIH NS061973

CRC/TR 43

GRK 1258

CRC/TR 128

CRC 1080

Title: Danger signals, IL-4 and neuroprotection after spinal cord injury

Authors: *J. WALSH[1,2,3], S. HENDRIX[8], F. BOATO[8,9], I. SMIRNOV[4,5], D. HECHLER[10], G. GOLZ[10], T. KAMMERTÖNS[11], J. VOIGT[12], C. VOGELAAR[12], V. SIFFRIN[12], A. RADJAVI[6,3,7], S. GADANI[1,3,2], A. FERNANDEZ-CASTANEDA[7,3], A. GAULTIER[7,3], R. GOLD[14], F. ZIPP[15], R. NITSCH[13], J. KIPNIS[3,7,6,2];
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Abstract: A body of experimental evidence suggests that T cells mediate neuroprotection following central nervous system (CNS) injury. Furthermore, T cells activated toward CNS-

specific antigens are particularly potent at neuroprotection, though the mechanism(s) underlying their beneficial effect are still unknown. Here we provide evidence that T cell-mediated neuroprotection after CNS injury can occur independently of canonical major histocompatibility class II (MHCII) signaling to T cell receptors (TCRs). This antigen-independent response is mediated by a MyD88-dependent Th2 induction through molecular mediators derived from the injured CNS tissue. The resulting IL-4 produced by CNS-infiltrating T cells directly protects injured neurons via signaling on neuronal IL-4 receptors. These findings shed a new light on the molecular mechanisms leading to a protective immune response to CNS injuries. Furthermore, it provides the first demonstration of a protective T-cell response induced by molecular signature of the injured tissue independent of MHCII-TCR interactions. These findings uncover novel mechanisms for T cell-mediated neuroprotection after CNS trauma, and can lead to the development of safe immune-based therapies in both CNS injury and neurodegenerative disorders.

Disclosures: **J. Walsh:** None. **S. Hendrix:** None. **F. Boato:** None. **I. Smirnov:** None. **D. Hechler:** None. **G. Golz:** None. **T. Kammertöns:** None. **J. Voigt:** None. **C. Vogelaar:** None. **V. Siffrin:** None. **A. Radjavi:** None. **S. Gadani:** None. **A. Fernandez-Castaneda:** None. **A. Gaultier:** None. **R. Gold:** None. **F. Zipp:** None. **R. Nitsch:** None. **J. Kipnis:** None.

Nanosymposium

286. Spinal Cord Injury: Therapeutic Strategies

Location: 152A

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 286.06

Topic: C.10. Trauma

Support: Canadian Institutes of Health Research

Title: Increased reactive sprouting in spinal cord-injured conditional Sox9 knockout mice

Authors: *A. BROWN[1], W. M. MCKILLOP[2], K. XU[2], T. HRYCIW[2];
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Abstract: Spinal cord injury (SCI) is a catastrophic event that is a major health care issue, causing lifelong disability. The absence of axonal regeneration after SCI has been attributed to axon-repelling molecules in the damaged myelin and in the scar. Amongst the most important inhibitory molecules in the scar are chondroitin sulfate proteoglycans (CSPGs) produced by reactive astrocytes responding to the injury. CSPGs are also key components of the perineuronal

matrix (PNNs) that surround the cell bodies and dendrites of many neurons in the central nervous system. PNNs stabilize synapses during development by preventing axonal sprouting onto inappropriate targets after appropriate connections have been made. Thus CSPGs may limit plasticity after SCI by inhibiting regeneration of axons through the glial scar or by inhibiting reactive sprouting at deafferented targets distant to the lesion. We have identified the transcription factor SOX9 as a key regulator of CSPG production in the injured spinal cord. We have also demonstrated reduced CSPG levels and improved locomotor recovery in spinal cord-injured *Sox9* conditional knockout mice. Herein we investigated sparing, long-range regeneration and reactive sprouting as possible explanations for the improved locomotor outcomes in *Sox9* knockout mice after SCI. Immunohistochemistry caudal to the lesion site demonstrates increased neuroplasticity in the *Sox9* knockout mice as indicated by increased levels of the presynaptic markers synaptophysin and vesicular glutamate 1 transporter (VGLUT1) and by increased serotonin immunoreactivity compared to controls. Whereas retrograde tract-tracing studies fail to reveal any evidence of increased sparing or of long-range regeneration in the *Sox9* knockout mice, anterograde tract-tracing experiments demonstrate increased reactive sprouting caudal to the lesion after SCI. The increased neuroplasticity and improved recovery after SCI in *Sox9* knockout mice highlights the clinical potential of *Sox9* antagonists as a treatment strategy for SCI.

Disclosures: A. Brown: None. **K. Xu:** None. **T. Hryciw:** None. **W.M. McKillop:** None.

Nanosymposium

286. Spinal Cord Injury: Therapeutic Strategies

Location: 152A

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 286.07

Topic: C.10. Trauma

Support: Canadian Paraplegic Association (Manitoba)

Health Sciences Foundation

Manitoba Medical Service Foundation

Title: Intracellular delivery of thioredoxin enhances neuroprotection in *in vitro* model of oxidative stress and *in vivo* model of spinal cord injury

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[1]Physiol., Regenerative Med. Group, and Spinal Cord Res. Cntr , Univ. of Mani, Winnipeg, MB, Canada; [2]Physiol., [3]Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: Increasing evidence indicate the importance of oxidation/reduction balance (Redox status) in cell biology. A sudden rise of oxidizing reactive oxygen species (ROS) levels after injury will quickly interrupt the redox status of the cell resulting in oxidation of vital proteins and initiation of cell death pathways. It is therefore hypothesized that enhancement of cell reducing capacity may have protective effects in oxidative stress related conditions. During the last two decades antioxidant therapies have been extensively used for treatment of neurotrauma buthave failed to generate optimal results in clinical applications. This indicates the need for novel treatment options. Thioredoxin (trx) is a 12kD protein with a central role in regulation of oxidative stress through maintaining the cellular proteins in reduced state. Intravenous administration of high doses of Trx has been shown to be protective in models of brain ischemia. Additionally, recent evidence indicates the ability of Trx for enhancing cell proliferation in adult brain-derived neural stem cells, suggesting its potential role for regeneration. The underlying protective mechanisms of intravenous trx therapy remain to be identified. Plasma Trx has a relative short half-life and does not cross the blood brain barrier (BBB) and therefore the extent of Trx tissue deposition after intravenous delivery is very minimal. In these studies we hypothesized that intracellular delivery of Trx will enhance the cell reducing capacity of neural cells and will improve neuroprotection. Methods: Routine molecular biology techniques were used to generate Trx constructs containing a cell-penetrating peptide. We used adult spinal cord cultures of neural stem cells to investigate the effect of intracellular delivery of Trx on cell proliferation. Neuroprotective effects were tested in cultures of SH-SY5Y and our clip compression model of spinal cord injury (SCI). Results: Our results indicate that our novel Trx intracellular delivery enhances Trx tissue deposition and distribution and enhances neuroprotection after SCI. Further details will be discussed. Conclusion: Our data indicate the efficacy of our novel Trx intracellular delivery as a potential therapeutic approach for treatment of neurotrauma.

Disclosures: E. Eftekharpour: None. **M. Iqbal:** None. **N. Pandian:** None.

Nanosymposium

286. Spinal Cord Injury: Therapeutic Strategies

Location: 152A

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 286.08

Topic: C.10. Trauma

Support: NIH/NIBIB Neuroengineering Training Grant 5T32EB003383-08

NSF DMR0748340

NSF Graduate Research Fellowship Program

Title: Local release of paclitaxel from aligned, electrospun poly(lactic-acid) microfibers promotes directed axonal extension

Authors: *J. A. ROMAN[1,4], A. HURTADO[2], H.-Q. MAO[4,3];
[1]Biomed. Engin., [2]Neurosci., [3]Materials Sci. and Engin., Johns Hopkins Univ., Baltimore, MD;
[4]Translational Tissue Engin. Ctr., Baltimore, MD

Abstract: Traumatic spinal cord injuries (SCIs) are characterized by an initial, physical insult followed by a secondary biological cascade, ultimately leading to the formation of a glial-lined cavity that inhibits axonal regeneration. Transplanting a scaffold of aligned, electrospun poly-lactic acid (PLA) microfibers into the cavity after a rat model of SCI promoted axonal regeneration. It has also been shown that axonal microtubule (MT) stabilization can be achieved by administering a low concentration of paclitaxel (PTX) to an SCI injury via an osmotic mini-pump. However, due to cerebral spinal fluid flow and the need for a persistent level of PTX, a higher dose was administered into the cavity, raising concerns about potential toxicity. By integrating the contact guidance with the ability for a prolonged release, we hypothesize that incorporating a sustained, local delivery of PTX from aligned PLA microfibers will promote axonal extension in a rat dorsal root ganglion (DRG) culture model. PTX was loaded into aligned PLA fibers at increasing loading concentrations (0, 0.05, 0.1, 0.5, 1.0, and 5.0% w/w PTX in reference to PLA weight) during the electrospinning process. The average diameter of these aligned fibers was 1.11 ± 0.14 μm. DRGs isolated from Sprague Dawley rats at P5 were cultured either on the different groups of fibers or with 10 nM of PTX in neurobasal media supplemented with nerve growth factor for five days. After fixation, cells were fluorescently labeled with an antibody against neurofilament, and the lengths of neurites were quantified for individual DRGs. Average neurite extension was calculated for each fiber sample ($n = 4 – 9$). Our data confirmed that a local release of PTX from electrospun PLA microfibers significantly promoted DRG neurite extension in comparison to PLA fibers alone or PTX supplemented to the media. Interestingly, low concentrations of PTX (0.05%) yielded significantly greater level of axonal extension than higher concentrations (5%), and neurite extension decreased as the concentration of PTX increased. Also, released PTX improved neurite extension compared to the same concentration of supplemented PTX ($p < 0.005$). These results show that locally released PTX effectively induces MT stabilization in a concentration-dependent manner. This local delivery of PTX from aligned microfibers can be easily integrated into nerve guidance conduits for future *in vivo* studies as well. Overall, a local release of PTX has promoted a more effective neurite extension than fibers or soluble PTX alone. This approach can potentially provide an effective

mechanism to promote axonal growth and regeneration after a traumatic central nervous system injury.

Disclosures: **J.A. Roman:** None. **A. Hurtado:** None. **H. Mao:** None.

Nanosymposium

286. Spinal Cord Injury: Therapeutic Strategies

Location: 152A

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 286.09

Topic: C.10. Trauma

Support: NSERC

MHRC

MMSF

HSCF

Title: Chondroitin sulfate proteoglycans negatively modulate the properties of adult spinal cord neural precursor cells by signaling through LAR and PTPσ receptors and activation of the Rho/ROCK pathway

Authors: ***S. M. DYCK**, A. ALIZADEH, E. PROULX, S. KARIMI-ABDOLREZAEE; Physiol., Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: Multipotent neural stem/progenitor cells (NPCs) reside in the spinal cord and are capable of replacing lost oligodendrocytes following spinal cord injury (SCI). Despite this intrinsic capacity, adult spinal cord NPCs mainly differentiate into astrocytes, with only a limited number becoming oligodendrocytes. This evidence emphasizes a key role for the post-SCI niche in modulating the regenerative response of spinal NPCs. We recently reported that injury-induced upregulation of chondroitin sulfate proteoglycans (CSPGs) potently restrict the survival, integration and differentiation of transplanted and endogenous NPCs in SCI. In vivo administration of chondroitinase (ChABC) promoted the long-term integration of transplanted NPCs and enhanced the activation and oligodendrocyte differentiation of resident NPCs after subacute and chronic SCI. Given the long-lasting upregulation of CSPGs in NPCs niche after SCI, it is important to unravel the potential mechanisms by which CSPGs influence the properties of NPCs. Using an in vitro model of the extracellular matrix of SCI, we investigated

the direct role of CSPGs on NPCs. In primary cultures of adult spinal NPCs, using cell viability, western blotting and immunocytochemistry assays, we show that CSPGs significantly decrease NPC growth and attachment, survival, proliferation and oligodendrocytes differentiation of adult spinal cord NPCs. Genetic down-regulation of CSPG receptors protein tyrosine phosphate receptor sigma (PTPσ) and leukocyte common antigen-related phosphatase (LAR) in NPCs attenuated the inhibitory effects of CSPGs on NPCs. CSPGs inhibitory effects were mediated through activation of the Rho/ROCK pathway and inhibition of Akt and Erk phosphorylation. Our data suggest the impact of CSPGs and its signaling receptors in governing the response of NPCs in their post-SCI niche, and identify new therapeutic targets for enhancing NPC-based therapies following SCI.

Disclosures: S.M. Dyck: None. **A. Alizadeh:** None. **E. Proulx:** None. **S. Karimi-Abdolrezaee:** None.

Nanosymposium

286. Spinal Cord Injury: Therapeutic Strategies

Location: 152A

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 286.10

Topic: C.10. Trauma

Title: Fumaric acid esters attenuate the early inflammatory response following spinal cord injury in mice via activation of the Nrf2 antioxidant pathway

Authors: E. ESPOSITO[1], I. PATERNITI[1], D. IMPELLIZZERI[1], M. CORDARO[1], *M.-A. SORTINO[2], S. CUZZOCREA[1];
[1]Dept. of Biol. and Envrn. Sci., Univ. of Messina, Messina, Italy; [2]Clin. Mol Biomedicine, Univ. of Catania, Catania, Italy

Abstract: Traumatic spinal cord injury (SCI) induces a long-standing inflammatory response in the spinal cord tissue, leading to a progressive apoptotic death of spinal cord neurons and glial cells. SCI is associated with severe disability and impairment in quality of life. Substantial experimental evidence supports reactive species as important mediators of secondary cell death after SCI. Early disease-modifying treatment options have mainly focused on inflammatory aspects of the disease. Fumaric acid esters (FAEs) are effective in patients with moderate to severe psoriasis. Recent studies also report the efficacy of one FAE component, dimethylfumarate (DMF), in relapsing forms of multiple sclerosis (MS). This study evaluated the effectiveness of FAEs to prevent cell loss and neurological dysfunction following compression to

the mice spinal cord. In vitro application of DMF led to stabilization of Nrf2, activation of Nrf2-dependent transcriptional activity and abundant synthesis of detoxifying proteins. On cellular levels, the application of FAE protected astrocytes against oxidative stress. Increased levels of Nrf2 were detected in spinal cord from DMF treated mice suffering from SCI. DMF reduced the severity of trauma induced by compression, the pro-inflammatory cytokine expression TNF-α, and improved the motor activity evaluated at 10 days post-injury. DMF treatment also decreased the expression of the apoptosis-associated markers BCL-2-associated X protein (BAX) and caspase 3, as well as the microglial cell markers OX42. Finally, Nrf2 is also up-regulated in the spinal cord from untreated mice with SCI, probably as part of a naturally occurring anti-oxidative response. In summary, anti-oxidative pathways are important players in SCI pathophysiology and constitute a promising target for future SCI therapies.

Disclosures: **E. Esposito:** None. **I. Paterniti:** None. **M. Sortino:** None. **D. Impellizzeri:** None. **M. Cordaro:** None. **S. Cuzzocrea:** None.

Nanosymposium

286. Spinal Cord Injury: Therapeutic Strategies

Location: 152A

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 286.11

Topic: C.10. Trauma

Support: Institut pour la Recherche sur la Moelle épinière et l'Encéphale (IRME)

Lyon Sciences Transfert. Univ-Lyon

Fondation pour le Recherche Médicale (FRM)

Title: Bio-scaffolding of axonal regeneration across the traumatic spinal cord

Authors: ***F. NOTHIAS**[1], J. CHEDLY[1], A. MONTEMBAULT[2], C. PESTRE[2], S. SOARES[1], Y. VON BOXBERG[1], L. DAVID[2];
[1]Team: Axon Regeneration and Growth, Neurosci. Paris Seine, CNRS-UMR8246, INSERM1130, Paris cedex 05, France; [2]IMP-ICE/ Univ. Claude Bernard Lyon 1, Villeurbanne, France

Abstract: Recent progress in the production of novel biomaterials offers a particularly promising perspective for the development of combinatorial therapeutic strategies for spinal cord injury (SCI) repair that will include implantation of such biomaterials into the lesion site, functioning

both as extracellular matrix substitute, and as bioactive support structure. Accordingly and as first step, we developed a therapeutic strategy based on the use of chitosan polymer, that exhibits ideal characteristics for tissue engineering. Thus, after evaluation of various structures, we were able to determine the formulation that appears the best suited for implantation into the SCI lesion site. Our experimental model is a thoracic dorsal hemisection in adult female rat, with or without implantation of polymer directly after the lesion. The bio-scaffolds lead to an important reduction of astrogliosis, impeding glial scar formation, tissue necrosis, and hence reducing the cavity formation. Astrocytic remodeling is accompanied by vigorous axon regrowth into the chitosan matrix, among which the cortico-spinal cord fibers. The regrowing axons are often found associated with astrocytic processes at the host-implant interface, and with newly formed, functional blood vessels colonizing the interior of the chitosan polymer. These results are evidence for the specific chitosan formulation creating a permissive, dynamic microenvironment for neural tissue regeneration.

Disclosures: **F. Nothias:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent# WO2014013188 A1. **J. Chedly:** None. **A. Montembault:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent# WO2014013188 A1. **C. Pestre:** None. **S. Soares:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); WO2014013188 A1. **Y. von Boxberg:** None. **L. David:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent# WO2014013188 A1.

Nanosymposium

286. Spinal Cord Injury: Therapeutic Strategies

Location: 152A

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 286.12

Topic: C.10. Trauma

Support: General Insurance Association of Japan

Title: Effect of combined therapy with neural stem cell transplantation and treadmill training for chronic spinal cord injury in mice

Authors: ***S. TASHIRO**[1], S. NISHIMURA[2], H. IWAI[2], G. ITAKURA[2], K. HORI[2], L. ZHANG[1], M. SHINOZAKI[3], A. IWANAMI[2], Y. TOYAMA[2], M. LIU[1], M. NAKAMURA[2], H.

OKANO[3];
[1]Dept. of Rehabil. Med., [2]Dept. of Orthopaedic Surgery, [3]Dept. of Physiol., KEIO Univ.School of Med., Tokyo, Japan

Abstract: [Background] Previous study demonstrated the effectiveness of neural stem-cell (NSC) transplantation in the sub-acute phase of spinal cord injury (SCI), but not in the chronic phase (Nishimura et al, Mol Brain 2012). It is partly because of the nature of microenvironment in chronically injured spinal cord and the long time disuse after SCI. Since treadmill training after SCI up-regulate the expression of neurotrophic factors and can modify the behavior affected by disuse, the treatment resistance could be, at least partially, overcome by training. Here, in this study, we investigated the effect of combination therapy with NSC transplantation and training for chronic SCI. [Method] In 30 adult C57/BL6J mice, severe contusive SCI was induced at T10 level using an IH-impactor. NSC transplantation was performed at 49 days post injury (DPI). Partial body weight supported bipedal gait treadmill training was performed from 42 to 105 DPI. The animals were randomly separated into following 4 groups: 1) NSC transplantation with training (TP-RH); 2) NSC transplantation without training (TP); 3) PBS injection with training (RH); and 4) PBS injection without training (Control). Locomotor function was assessed with Basso Motor Scoring scale, and survival of the transplanted cell was evaluated with bio-imaging (IVIS spectrum system) up to 133 DPI. Side effects of intervention(s) on spasticity and allodynia were also assessed with strain-gauge test and with von-Frey and Hargreaves tests. Immunohistological characterization of transplanted cell differentiation was performed at 133 DPI. [Result] Transplanted cells were well survived and differentiated into neurons, astrocytes, and oligodendrocytes. The mice of the TP-RH group showed significantly better functional recovery compared to the other 3 groups. Regarding the side effects, there were no significant differences in each of spasticity and allodynia test among the four groups. [Discussion] The locomotor function was significantly recovered when stem-cell therapy was combined with rehabilitation without any adverse effect. This result would be achieved via several mechanisms, such as the re-organization of neuronal network (Zhang et al, Mol Brain 2014), the up-regulation of neurotrophic factors and the overcoming of disuse. Further investigation is needed to clarify the background of this synergistic effect of NSC transplantation and rehabilitation for chronic SCI.

Disclosures: **S. Tashiro:** None. **S. Nishimura:** None. **H. Iwai:** None. **G. Itakura:** None. **K. Hori:** None. **L. Zhang:** None. **M. Shinozaki:** None. **A. Iwanami:** None. **Y. Toyama:** None. **M. Liu:** None. **M. Nakamura:** None. **H. Okano:** None.

Nanosymposium

286. Spinal Cord Injury: Therapeutic Strategies

Location: 152A

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 286.13

Topic: D.13. Motor Neurons and Muscle

Title: Use of perfluorocarbon (oxycyte) as innovative therapy for spinal cord injury

Authors: *S. BOURIKIAN[1], A. YACOUB[2], B. MATHERN[3], H. YOUNG[2];
[1]VCU Sch. of Med., Richmond, VA; [2]Neurosurg., VCU Heath Syst., Richmond, VA;
[3]Neurosurg., VCU Hlth. Syst., Richmond, VA

Abstract: The goal of perfluorocarbon (Oxycyte) emulsion in a traumatic Spinal Cord Injury (SCI) model is the reduction of hypoxia and the interruption of the cascade of secondary injury following the initial injury. This secondary injury leads to necrotic and apoptotic death of neurons and glia, and subsequent demyelination which could be irreversible and lead to permanent neurological deficits and long-term disability. Although the primary injury can't be reversed, steps can be taken to reduce and ultimately attempt to prevent this secondary injury cascade. The most critical role in the progression of the secondary injury is played by hypoxia at the site of injury. To minimize the deleterious effects of hypoxia, it is critical to efficiently deliver oxygen to the damaged cord region, not only to act as a form of treatment but also as a method of further injury prevention. Using a perfluorocarbon (Oxycyte) infusion for this process is ideal due to its very small particle size (<0.2 μm) and highly efficient O_2 solubility. This study evaluates the efficacious dose of Oxycyte infusion on neuronal and tissue preservation, and retention of motor and cognitive capability in animals after SCI. The therapeutic value of Oxycyte at 2 ml/kg and 5 ml/kg was evaluated. We hypothesized that animals treated with different Oxycyte doses will exhibit different levels of reduced neuronal and tissue damage as well as different levels of motor and sensory functions after SCI. We found that the Basso, Beattie, and Bresnahan(BBB) scale and inclined plane test of both low and high dose Oxycyte treated animal groups were significantly higher as compared to the vehicle group after spinal cord injury ($P < 0.05$). Moreover, the cavity volume was significantly reduced in the Oxycyte group ($P = 0.039$) and Oxycyte significantly preserved white matter ($P= 0.0076$) as compared to the control group. Furthermore, the Oxycyte group had significantly less apoptotic neuronal cell death rostral and caudal to the lesion epicenter ($P= 0.0072$ and $P= 0.0033$) respectively. The functional recovery score and neuronal protective values of 2 ml/kg Oxycyte treated group were lower (BBB score 11 ± 1.28) compared to (BBB score 14.6 ±1.05) the 5 ml/kg group. This suggests that searching for the optimum therapeutic dose is warranted.

Disclosures: **S. Bourikian:** None. **A. Yacoub:** None. **B. Mathern:** None. **H. Young:** None.

Nanosymposium

286. Spinal Cord Injury: Therapeutic Strategies

Location: 152A

Time: Monday, November 17, 2014, 8:00 AM - 11:30 AM

Presentation Number: 286.14

Topic: C.10. Trauma

Support: Abbvie

Krembil Neuroscience Program

Title: Effect of RGM neutralizing antibody on functional recovery following spinal cord injury

Authors: *A. J. MOTHE[1], R. PENHEIRO[1], A. SHABANZADEH[1], P. MONNIER[1], B. K. MUELLER[2], C. H. TATOR[1,3];
[1]Div. Genet. & Develop., Krembil Discovery Tower, Toronto Western Hospit, Toronto, ON, Canada; [2]GmbH & Co KG Knollstrasse, Abbvie Germany, Ludwigshafen, Germany; [3]Surgery, Univ. of Toronto, Toronto, ON, Canada

Abstract: Spinal cord injury (SCI) is a devastating condition with great personal and societal costs. Currently, there is no effective treatment for major SCI. Following the initial trauma, there is a cascade of molecular and degenerative events including apoptosis, ischemia, and the upregulation of inhibitory molecules which prevent regeneration and limit recovery. Repulsive guidance molecule (RGMa) is a potent inhibitor of axonal growth in both the developing and adult CNS. After SCI, traumatic brain injury, and ischemia, RGMa is upregulated in myelin and the scar tissue surrounding the lesion. RGMa expression inhibits axon regeneration following injury, an effect that can be neutralized by RGMa antibody administration. We used human RGMa monoclonal antibodies (mAbs) specifically targeting RGMa in a clinically relevant animal model of acute impact-compression SCI. RGMa mAbs were administered intravenously immediately following injury at thoracic level T8 in adult rats and weekly until sacrifice at 6 weeks post-SCI. Functional and sensitivity tests were performed and biotinylated dextran amine (BDA) was injected into the sensorimotor cortex for anterograde tracing of the corticospinal tract, which is completely interrupted with this severity of injury. Rats receiving mAb treatment showed significant improvement in the BBB open field locomotor score, and RGMa mAbs also attenuated mechanical and thermal allodynia. Some BDA labeled fibers were also apparent caudal to the lesion in RGMa treated rats. Immunostaining for glial reactivity and quantitation of perilesional neurons is ongoing. In summary, these results show that RGMa neutralizing antibody promotes axonal regeneration/sprouting and functional recovery after SCI.

Disclosures: **A.J. Mothe:** None. **R. Penheiro:** None. **A. Shabanzadeh:** None. **P. Monnier:** None. **B.K. Mueller:** A. Employment/Salary (full or part-time):; Abbvie. **C.H. Tator:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Abbvie.

Nanosymposium

287. Mood Disorders: Novel Therapeutic Mechanisms

Location: 140A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 287.01

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH/NIMH Grant R01 MH092412

Title: Antidepressant-like effects of the k-opioid receptor partial agonist nalmefene

Authors: ***C. A. BROWNE**, I. LUCKI;
Psychiatry, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Treatment resistant depression (TRD) is increasingly common among severely ill major depressed patients. There is a substantial medical need to develop novel, rapid-acting antidepressants. Activation of the dynorphin/kappa opioid receptor (DYN/ κ-OR) system is implicated in the development of depression. κ-OR antagonists produce rapid and long-lasting antidepressant-like effects in preclinical animal studies. However, most of these drugs are unsuitable for clinical development. Nalmefene is a κ-OR partial agonist and is medically available after being licensed in Europe for alcohol use disorder. However, the potential of nalmefene to treat affective disorders has not been previously assessed. Therefore, we characterized the effects of nalmefene in rodent behavioral tests for depression and antidepressant responses. Protocols were conducted in accordance with the NIH Guide for Care and Use of Laboratory Animals. We first characterized the behavioral effects of nalmefene using the FST in the Wistar Kyoto (WKY) rat, a genetic model of pathological anxiety and depression. WKY rats exhibited dose-dependent decreases in immobility levels in the FST following nalmefene treatment. Next, nalmefene was examined using female C57BL/6J and mice with genetic deletion of κ-OR (OprK1-/- mice). Nalmefene significantly reduced the immobility scores of C57BL/6J mice, but not of OprK1-/- mice, in the FST. These data indicate that κ-ORs mediate the antidepressant-like effects of nalmefene in mice. These are the first data to indicate that nalmefene produces antidepressant-like effects in the FST using two rodent models, likely by modulating κ-ORs. Nalmefene's novel antidepressant-like qualities may have potential for use as a therapeutic in TRD.

Disclosures: **C.A. Browne:** None. **I. Lucki:** None.

Nanosymposium

287. Mood Disorders: Novel Therapeutic Mechanisms

Location: 140A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 287.02

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: AA is supported by the government of Saudi Arabia

Title: The antidepressant-like effects of combination buprenorphine/naltrexone in the novelty-induced hypophagia task in mice

Authors: ***S. J. BAILEY**, A. ALMATROUDI, C. P. BAILEY, S. M. HUSBANDS;
Dept. of Pharm. and Pharmacol., Univ. of Bath, Bath, United Kingdom

Abstract: Antagonists at kappa-opioid receptors have been proposed as novel antidepressants. The standard high-affinity, selective kappa-antagonists have a long lasting duration of action which potentially limits their use (Carroll and Carlezon 2013. J Med Chem 56: 2178-2195). Buprenorphine is a partial mu-opioid receptor agonist and a kappa-antagonist, while naltrexone is a non-selective opioid antagonist. We have previously shown that the combination of buprenorphine (1mg/kg) with naltrexone (1mg/kg) produced a functional short-acting kappa-antagonist that was non-sedating and non-rewarding in mice. Here, we report the effects of this combination treatment on depression-related behaviour. Adult male CD-1 mice (8-9 weeks) were used. For novelty-induced hypophagia, mice were individually housed and trained for 3 days to consume condensed milk. On test days, mice were injected intraperitoneally (10 ml/kg) with saline, buprenorphine alone (1 mg/kg), naltrexone alone (1 mg/kg), buprenorphine/naltrexone combination (1 mg/kg), norBNI (10mg/kg) or fluoxetine (20 mg/kg) one hour prior to testing behaviour. The latency to drink and consumption were recorded in the home cage (day 4) and in the novel cage (day5). One-way ANOVA, revealed that there was a significant effect of drug treatment on the latency to drink in the novel cage ($F_{(5, 54)} = 8.5$, $P<0.001$) but not consumption ($F_{(5, 54)} = 1$, $P=0.4$). The combination of buprenorphine/naltrexone produced antidepressant-like effects, significantly reducing the latency to drink in the novel cage, compared with controls. Interestingly, the effects of the combination treatment were similar to naltrexone alone. We have previously shown that mixed mu-/kappa-opioid receptor antagonists have antidepressant and anxiolytic potential (Casal-Dominguez et al. 2013 J Psychopharm 27:192-202). We are

investigating whether combination buprenorphine/ naltrexone may also have anxiolytic potential in mice.

Disclosures: S.J. Bailey: None. **A. Almatroudi:** None. **C.P. Bailey:** None. **S.M. Husbands:** None.

Nanosymposium

287. Mood Disorders: Novel Therapeutic Mechanisms

Location: 140A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 287.03

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Avanir Pharmaceuticals

West Virginia University

Title: Preclinical evaluation of the fast acting antidepressant potential of dextromethorphan: Involvement of AMPA and sigma-1 receptors

Authors: *L. NGUYEN, R. R. MATSUMOTO;
Basic Pharmaceut. Sci., WVU Sch. of Pharm., Morgantown, WV

Abstract: Accumulating evidence indicates that sub-anesthetic doses of ketamine can exert robust and rapid antidepressant effects even in treatment-resistant individuals, but its widespread use remains limited by its abuse liability and adverse effects. Thus, in the present preclinical study, the over-the-counter antitussive dextromethorphan was investigated as a potential alternative to ketamine due to its overlapping pharmacodynamic properties. In addition, the role of AMPA and sigma-1 receptors in the antidepressant-like actions of dextromethorphan was examined because mounting evidence suggests that these mechanisms may contribute to a fast onset of antidepressant efficacy. Our results revealed for the first time that administration of dextromethorphan to male Swiss, Webster mice produces antidepressant-like effects in a dose-dependent manner in both the forced swim and tail suspension tests, similar to the positive controls for fast acting and conventional antidepressant effects characterized by ketamine and imipramine, respectively. Moreover, concomitant administration of quinidine (CYP2D6 inhibitor) to decrease the first-pass metabolism of dextromethorphan potentiated its antidepressant-like actions, revealing dextromethorphan itself has antidepressant efficacy. Finally, using the forced swim test, the most validated animal model for predicting

antidepressant efficacy, pretreatment of mice with a behaviorally inactive dose of NBQX (AMPA antagonist) or BD1063 (sigma-1 antagonist) significantly blocked the antidepressant-like actions of dextromethorphan, indicating that AMPA and sigma-1 receptors play pivotal roles in mediating the antidepressant-like properties of dextromethorphan. Together, the data show that dextromethorphan exerts antidepressant-like actions through both AMPA and sigma-1 receptors, strongly suggesting that dextromethorphan should be further explored for translational potential as a safe and effective fast acting antidepressant.

Disclosures: L. Nguyen: None. **R.R. Matsumoto:** 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.; Avanir Pharmaceuticals. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Avanir Pharmaceuticals. F. Consulting Fees (e.g., advisory boards); Avanir Pharmaceuticals.

Nanosymposium

287. Mood Disorders: Novel Therapeutic Mechanisms

Location: 140A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 287.04

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NICHHD T32HD07430

R37 MH068542

R01 AG043688

T32 MH015174-36

DP5 OD017908-01

HDRF

NYSTEM

Title: A single injection of ketamine confers robust, long-term protection against stress-induced depressive-like behaviors

Authors: *R. A. BRACHMAN, R. HEN, C. A. DENNY;
Dept. of Neurosci., Columbia Univ., New York, NY

Abstract: Stress exposure is one of the greatest risk factors for psychiatric illnesses like Major Depressive Disorder (MDD) and Post-Traumatic Stress Disorder (PTSD). However, not all individuals exposed to stress develop affective disorders. Stress resilience, the ability to experience stress without developing persistent psychopathology, varies from individual to individual. Enhancing stress resilience in at-risk populations could potentially protect against stress-induced psychiatric disorders. Despite this fact, no resilience-enhancing pharmaceuticals have yet been identified. Using a chronic social defeat stress model in mice, we tested if ketamine, a novel, rapid-acting antidepressant, could protect against depressive-like behavior. Mice were administered a single sub-anesthetic dose of ketamine and then one week later were subjected to two weeks of social defeat. Social defeat robustly and reliably induced depressive-like behavior. Mice treated with prophylactic ketamine were protected against the deleterious effects of SD in the forced swim test and in the social interaction test. Stress resilience induced by a single injection of ketamine was robust and long lasting - persisting over a period of prolonged stress exposure and at least 3 weeks post-injection. As the half-life of ketamine is only a few hours, ketamine was not on-board at any point during the 2-week stress paradigm. These data suggest that ketamine can induce persistent stress resilience and may, therefore, be used to protect against stress-induced MDD and PTSD. To our knowledge, this is the first study to examine the potential of a clinic-ready drug to provide long-term prophylactic protection against the induction of stress-related disorders. This offers a new approach to protecting at-risk populations against stress-induced pathology.

Disclosures: R.A. Brachman: None. **R. Hen:** F. Consulting Fees (e.g., advisory boards); Lundbeck, Servier, Roche. **C.A. Denny:** None.

Nanosymposium

287. Mood Disorders: Novel Therapeutic Mechanisms

Location: 140A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 287.05

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH 1F31MH100842

NIMH 1K01MH099371

NIMH R37MH068542

NYSTEM C024330

Hope for Depression Research Foundation

Title: The contribution of NR2B-dependent plasticity in adult-born granule cells to antidepressant action

Authors: *L. TANNENHOLZ, R. HEN, M. A. KHEIRBEK;
Columbia Univ., New York, NY

Abstract: Neurogenesis ablation studies have shown that adult-born granule cells (GCs) are necessary for some of the behavioral responses to antidepressants (ADs) seen in rodents. Yet, the mechanism by which newborn neurons contribute to AD action remains unknown. During the maturation process, immature neurons exhibit unique properties that could underlie their ability to influence behavioral output. In particular, adult-born GCs in the DG exhibit a period of heightened plasticity 4 to 6 weeks post-mitosis which is mediated by NR2B-expressing NMDA receptors. The functional contribution of this critical window to AD responsiveness is unknown. Here, we used an inducible transgenic mouse line to specifically delete NR2B-containing NMDA receptors on adult-born GCs and ran the mice in a number of anxiety and depression-related behavioral assays following chronic treatment with the AD, fluoxetine (18mg/kg/day). In the tail suspension test we observed that fluoxetine lowered immobility regardless of the presence or absence of NR2B. However, in the novelty suppressed feeding test - which is a behavioral paradigm in which the efficacy of fluoxetine is neurogenesis-dependent - we found that deletion of NR2B attenuated the decrease in latency to feed seen after chronic fluoxetine treatment. This indicates that one mechanism by which FLX produces an anxiolytic effect in the NSF test is by increasing the number of highly plastic units in the DG circuit. Currently, we are examining whether the neurogenic effects of fluoxetine are preserved in mice lacking NR2B in adult-generated GCs.

Disclosures: L. Tannenholz: None. **R. Hen:** F. Consulting Fees (e.g., advisory boards); Lundbeck, Servier, Roche. **M.A. Kheirbek:** None.

Nanosymposium

287. Mood Disorders: Novel Therapeutic Mechanisms

Location: 140A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 287.06

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Inhibition of phosphodiesterase 2 reverses stress-induced mood disorders: Involvement of nadph oxidase functions

Authors: *Y. XU[1], L. RUAN[1], X. XIE[1], H. ZHANG[2], J. M. O'DONNELL[1];
[1]Dept. of Pharmaceut. Sci., State Univ. of New York at Buffalo, Buffalo, NY; [2]West Virginia Univ., Morgantown, WV

Abstract: The correspondence between the prevalence of exposure to oxidative stress and the development of depression/anxiety is lacking. Chronic stress alters the expression of genes affecting redox state, such as excess levels of reactive oxygen species (ROS) and reduced superoxide dismutase (SOD) expression, which result in functional abnormalities in the hippocampus. Phosphodiesterase 2 (PDE2) is highly expressed in the hippocampus, which plays an important role in both the development and the treatment of stress-related depression and anxiety. This study investigated the relationship between PDE2 activity and the ROS expression in the hippocampus after stress and how PDE2 inhibitor and PDE2 silence regulated stress-induced depression- and anxiety-like behaviors through affecting redox state. The first set of study investigated the effects of PDE2 inhibitor Bay 60-7550 on depression- and anxiety-like behaviors in tail suspension, forced swimming, marble bury and novelty suppressed feeding tests in chronically stressed mice. Pretreatment with the oxidizing agent DTNB prevented, while the reducing agent DTT potentiated, the effects of Bay 60-7550 on behaviors, further supporting the relationship between PDE2 activity and the oxidative stress-induced depression and anxiety. Silencing PDE2 by microinfusion of lentiviral vectors expressing shRNAs that targeted PDE2A into bilateral hippocampal CA1 subregions before chronic stress produced antidepressant- and anxiolytic-like effects. These effects were prevented by pretreatment with DTNB and ameliorated by DTT. The subsequent study suggested that oxidative stress-induced ROS expression was positively related to PDE2 levels, which was consistent with the in *vivo* data. PDE2 inhibitor Bay 60-7550 decreased stress hormone corticosterone-induced increases in NADPH oxidase subunits, such as p47 phox, p67 phox and gp91 phox, in the hippocampal cells. Pretreatment with the antibodies of the p47 phox, p67 phox and gp91 phox prevented the effects of Bay 60-7550 on the expression of the brain derived neurotrophic factor (BDNF). The present results provide the evidence that PDE2 inhibition may represent a novel therapeutic target for stress-related psychiatric disorders, such as depression and anxiety. Key words: Stress; PDE2; Corticosteron; Depression; Anxiety; NADPH Oxidase

Disclosures: **Y. Xu:** None. **L. Ruan:** None. **X. Xie:** None. **H. Zhang:** None. **J.M. O'Donnell:** None.

Nanosymposium

287. Mood Disorders: Novel Therapeutic Mechanisms

Location: 140A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 287.07

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant MH077159

Title: Angiotensin II type I receptor antagonist azilsartan reduces emotionality in mice exposed to unpredictable chronic mild stress

Authors: *J. N. PARRISH[1,2,4], M. SENEY[1,2], S. PIANTADOSI[1,2,4], B. FRENCH[1,2], H. OH[1,2,4], A. SVED[1,3], E. SIBILLE[1,2];
[2]Dept. of Psychiatry, [3]Dept. of Neurosci., [1]Univ. of Pittsburgh, Pittsburgh, PA; [4]Ctr. for Neurosci., Pittsburgh, PA

Abstract: A bidirectional link has been reported between cardiovascular disease (CVD) and major depressive disorder (MDD). Although clinical studies suggest that angiotensin II type I receptor blockers (ARBs) may have antidepressant-like effects, this has not been thoroughly investigated. This study investigates whether azilsartan, a long-lasting ARB, can reduce anxiety and depressive-like behavior (emotionality) in mice exposed to unpredictable chronic mild stress (UCMS). C57BL6/J female mice were divided into treatment groups (non-stressed+vehicle, UCMS+vehicle, UCMS+0.5mg/kg/day azilsartan). After an initial 3-week exposure to UCMS, mice received drug treatment for 8 weeks (during continuous UCMS). Behavioral testing was performed, and test scores were integrated in emotionality z-scores. Non-stressed vehicle-treated mice spent more time in the open arms of the elevated plus maze than UCMS vehicle-treated mice ($p<0.05$). In the novelty suppressed feeding test, UCMS mice treated with vehicle took longer to bite the pellet than non-stressed vehicle-treated mice ($p<0.05$), and there was a trend for UCMS vehicle-treated mice to take longer to bite the pellet than UCMS azilsartan-treated mice ($p<0.1$). In the cookie test, UCMS azilsartan-treated mice took less time to bite the cookie than UCMS vehicle-treated mice ($p<0.01$). When all behavioral tests were combined into an emotionality z-score, UCMS vehicle-treated mice had higher emotionality (i.e. more anxiety-/depressive-like behavior) than non-stressed vehicle-treated mice ($p<0.001$) and UCMS azilsartan-treated mice ($p<0.05$). Azilsartan is effective at reducing emotionality in female mice exposed to UCMS. Future studies will investigate the time trajectory for potential fast-acting antidepressant activity and site of action.

Disclosures: **J.N. Parrish:** None. **M. Seney:** None. **S. Piantadosi:** None. **B. French:** None. **H. Oh:** None. **A. Sved:** None. **E. Sibille:** None.

Nanosymposium

287. Mood Disorders: Novel Therapeutic Mechanisms

Location: 140A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 287.08

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIDA Grant T32DA007255

NIMH Grant R01MH079103

Title: GLO1 inhibition as a novel fast-acting antidepressant

Authors: ***K. M. MCMURRAY**, A. A. PALMER;
Human Genet., Univ. of Chicago, Chicago, IL

Abstract: Major depressive disorder (MDD) is a highly prevalent and extremely debilitating mental disorder. Current treatments for MDD are limited by the slow onset of therapeutic effects (2-4 weeks), adverse side effects (weight gain, insomnia) or a lack of response to treatment in a large portion of patients (~50%). Thus, the identification of novel systems and molecular targets for treatment is of paramount importance. We have recently identified Glyoxalase I (GLO1) as a novel target for the treatment of depression. GLO1 is a ubiquitous cellular enzyme responsible for the detoxification of methylglyoxal (MG), which is a byproduct of glycolysis. We recently showed that MG is a competitive partial agonist at GABA-A receptors and that inhibition of GLO1 both increases MG concentrations within the brain and reduces anxiety-like behaviors in mice. Clinical studies of GABA-A receptor agonists have shown that they are not effective treatments for depression. However, previous studies have identified correlations between Glo1/MG and depression-like behavior in mice. Thus, we investigated the effects of both pharmacological and genetic manipulation of GLO1 on depression-like behavior in mice. The tail suspension test (TST) and forced swim test (FST) are assays of antidepressant efficacy. Mice treated acutely with antidepressants spend more time performing escape-oriented behaviors than immobile postures. We found that both genetic and pharmacological inhibition of GLO1 (using pBBG, which is a small molecule inhibitor of Glo1) reduced immobility in the TST and FST. In these tests, GLO1 inhibition reduced immobility in both male and female mice across multiple mouse strains (C57BL/6J, BALB/cJ and FVB/nJ; $p<0.05$) without affecting locomotor behavior

in the open field test (OFT). The chronic FST selectively respond to chronic treatments (14 day) with classical antidepressants (Fluoxetine; SSRI) mimicking the clinical time-course of antidepressant onset in humans. Mice were tested in the chronic FST to establish a time-course of therapeutic onset for GLO1 inhibitors. We found that pharmacological inhibition of GLO1 by pBBG reduced depression-like behavior with classical antidepressants to a similar extent as fluoxetine by 14 days of treatment ($p<0.05$). Further, pBBG reduced depression-like behavior by 5 days of treatment suggesting that GLO1 inhibitors may be novel fast-acting antidepressants.

Disclosures: K.M. McMurray: None. **A.A. Palmer:** None.

Nanosymposium

287. Mood Disorders: Novel Therapeutic Mechanisms

Location: 140A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 287.09

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

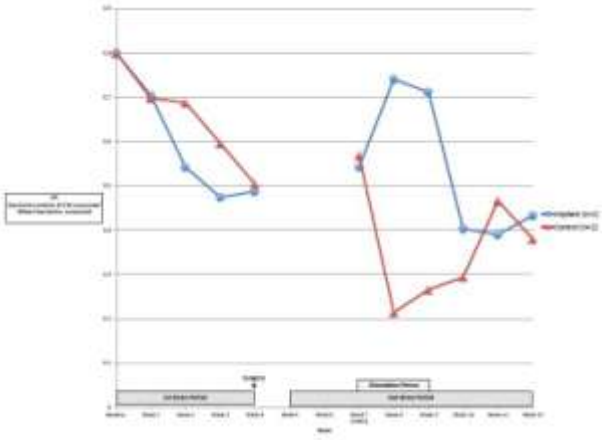
Support: Vivian Smith Department of Neurosurgery Research Fund, University of Texas

Title: Deep Brain Stimulation of the Median Forebrain Bundle reverses anhedonia in a chronic stress model of depression

Authors: P. COTTON[1], *A. J. FENOY[2,1];
[1]Neurosurg., Univ. of Texas, Houston, TX; [2]Neurosurg., Mischer Neurosurgical Associates, Houston, TX

Abstract: Study Objective Deep brain stimulation (DBS) is a neurosurgical treatment modality utilized in a variety of diseases. Some patients who have undergone subthalamic nucleus stimulation have experienced hypomania, and it has been hypothesized that this may be due to inadvertent activation of the median forebrain bundle (MFB). This has prompted some groups to explore this target as a therapeutic option for patients with treatment resistant depression (TRD). As far as we know there is not an animal model to parallel the human studies. Our purpose is to create a suitable model of depression, implant an electrode within the MFB and determine the role stimulation plays in changing behavior. **Methods** In order to emulate depression a chronic unpredictable stress (CUS) model was utilized to produce anhedonia. Male Sprague Dawley rats were exposed to an assortment of daily stresses that repeats on a weekly schedule. The efficacy of the stress program was measured by following a decline in the rat's preference for a sweet solution over water (sweetness preference index, SPI). CUS was employed for 4 weeks, then

either a stereotaxic implant of an electrode to the left MFB or a sham surgery was performed. Stress resumed after 1 week recovery. During post surgical weeks 3 to 5, a daily 6-hr period of DBS was added to the CUS program. CUS continued for 3 additional weeks before rats were sacrificed. **Results** The CUS program induced a decrease in SPI. Stimulation of the MFB nearly reversed the anhedonic state of the stimulated rats as measured by the SPI ($p<0.001$), whereas the sham group showed persistence of SPI suppression. With cessation of stimulation the SPI returned to levels comparable to the sham counterparts. **Conclusions** Under the paradigm of CUS/SPI model the anhedonia experienced by the rats was reversed during the stimulation phase of the experiment. This exciting finding validates the early results seen when using this target for TRD in humans. Further inquiry is needed to define the possible neurochemical mediator(s) and the role(s) played in involved circuits.



Disclosures: **P. Cotton:** None. **A.J. Fenoy:** None.

Nanosymposium

287. Mood Disorders: Novel Therapeutic Mechanisms

Location: 140A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 287.10

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Dbs of the bnst for treatment resistant ocd

Authors: *L. ISLAM[1,3], R. RANIERI[1,3], G. MESSINA[4], A. FRANZINI[4], S. SCARONE[1,3], O. GAMBINI[2,3];

[1]Psychiatry, [2]Ospedale San Paolo, Milan, Italy; [3]Univ. of Milan, Milan, Italy; [4]Inst. Neurologico Carlo Besta, Milan, Italy

Abstract: Introduction: Obsessive compulsive disorder (OCD) is a highly disabling condition which can lead to severe impairment in social, cognitive and interpersonal functioning. Deep-brain stimulation (DBS) has been demonstrated to be an effective and safe interventional procedure in refractory forms in selected cases. Several surgical targets have been proposed, including the anterior limb of internal capsule (ALIC) and the Nucleus Accumbens (NAc). The Bed Nucleus of the Stria Terminalis (BNST) has recently been described as a potentially successful target for the surgical treatment of OCD. **Subjects and Methods:** We describe 2 patients suffering from severe, chronic, drug-resistant OCD, who underwent DBS of the BNST. Patients were selected because of chronic(i.e. duration of illness greater than 5 years), severe (YBOCS score of 30 or more) drug resistant OCD (at least 2 trials of SSRIs, 1 trial with clomipramine, augmentation with antipsychotics and/or CBT). The effects of DBS for OCD were examined in our patients in a short-term, unblended follow-up. After preoperative imaging with MRI and CT scans, images were merged to obtain the coordinates of the BNST. Patients underwent insertion of electrodes, and during the same surgical session two pulse generators were implanted in the subcutaneous tissue of subclavian region on each side for both patients. The generators were activated four days after surgery at an amplitude of 0.5 V bilaterally, 130 Hz frequeny and 210 msec duration of impulse. The amplitude was slowly increased (by average 0.5 V every two weeks) for both patients, until an amplitude of 4.5 V was achieved. YBOCS ratings were performed at weekly visits. Medication was not withdrawn. **Results:** Both patients had a significant decrease in YBOCS scores at the one month follow up visit (-5 points for patient 1, -10 points for patient 2), at the three month follow up (-7 points from baseline for patient 1, -12 points from baseline for patient 2) and at the 6 months follow up visit (-8 points for patient 1, -14 points for patient 2) **Conclusions:** Both patients showed a significant overall improvement in OCD symptoms after DBS of the BNST.

Disclosures: L. Islam: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); medtronic. **R. Ranieri:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); medtronic. **G. Messina:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); medtronic. **A. Franzini:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); medtronic. **S. Scarone:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); medtronic. **O. Gambini:** 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.

Nanosymposium

287. Mood Disorders: Novel Therapeutic Mechanisms

Location: 140A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 287.11

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH/NIMH Grant R01 MH086599

NIH/NIMH Grant T32 MH14654

Title: Corticosteroid treatment increases sensitivity to behavioral effects of fluoxetine in C57BL/6J mice

Authors: *S. A. ROBINSON[1,2], B. R. BROOKSHIRE[3], I. LUCKI[3];
[2]Neurosci., [3]Psychiatry, [1]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Antidepressant treatment with selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, has been shown to increase hippocampal neurogenesis and ameliorate depressive-like behaviors in certain strains of mice. The C57BL/6J mouse strain, however, is known to be relatively insensitive to the neurogenic and behavioral effects of chronic antidepressant treatments. Previous studies in our lab have shown that chronic co-administration of corticosterone (CORT) and fluoxetine produce proliferative effects in the hippocampus of C57BL/6J mice. In this study, we examined whether prolonged exposure to CORT would also alter the behavioral response of C57BL/6J to chronic treatment with the SSRI antidepressant fluoxetine. For this study we implemented two methods of CORT exposure. One cohort of C57BL/6J mice was surgically implanted with subcutaneous pellets that continuously released CORT (20 mg/kg/day) or placebo for 21 days. The other received CORT orally via drinking water at a dose of 35 µg/ml or vehicle for 21 days. For the last 14 days of CORT exposure both cohorts were injected concurrently with fluoxetine (5 mg/kg, i.p., b.i.d.) or saline. Animals co-treated with pellet CORT and fluoxetine showed a significant reduction in latency to approach food in the novelty-induced hypophagia (NIH) test compared to controls ($p<0.01$, n=9-10/group). Animals treated with oral CORT and fluoxetine also displayed a significant decrease in latency to approach compared to controls ($p<0.001$, n=20/group). Interestingly, animals treated with oral CORT and fluoxetine showed no significant changes in hippocampal cell proliferation, as measured by flow cytometry-detected BrdU labeling, suggesting that this behavioral effect may be independent of alterations in neurogenesis. Our findings show that CORT exposure increases sensitivity to the anxiolytic effects of fluoxetine in C57BL/6J. These

effects may be mediated by the synergistic interaction of serotonin with glucocorticoid and mineralocorticoid receptors.

Disclosures: **S.A. Robinson:** None. **B.R. Brookshire:** None. **I. Lucki:** None.

Nanosymposium

287. Mood Disorders: Novel Therapeutic Mechanisms

Location: 140A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 287.12

Topic: E.05. Stress and the Brain

Support: Department of the Army DMRDP W81XWH-11-2-0077

Title: Paroxetine modulates Nociceptin/Orphanin FQ and partially reverses mechanical and thermal allodynia in an animal model of post-traumatic stress disorder and chronic pain

Authors: P. DIB[1], Y. ZHANG[1], C. D. SIMPSON-DURAND[1], *K. M. STANDIFER[1,2,3]; [1]Pharmaceut. Sci., Univ. of Oklahoma HSC Col. of Pharm., OKLAHOMA CITY, OK; [2]Cell Biol., Col. of Med., Univ Oklahoma HSC, OK; [3]Oklahoma Ctr. for Neurosci., Univ. of Oklahoma HSC, Oklahoma City, OK

Abstract: Single-prolonged stress (SPS) is an established animal model for post-traumatic stress disorder (PTSD). We have previously reported that SPS induces long-lasting mechanical allodynia and thermal hyperalgesia, hypocortisolism, and elevated Nociceptin/Orphanin FQ (N/OFQ) in serum and cerebrospinal fluid. In humans, the selective serotonin reuptake inhibitor, Paroxetine (PRX), is indicated as a first line treatment for PTSD. Since many PTSD patients also suffer from chronic pain, the aim of this study was to determine the ability of PRX to reverse the allodynia, hyperalgesia and other changes resulting from SPS. To achieve this, male Sprague-Dawley rats were subjected to SPS; half of the SPS and control group rats were immediately presented with PRX treatment in drinking water (0.03 mg/ml) daily for 21 or 28 days, respectively. PRX attenuates the mechanical and thermal allodynia associated with SPS ($p<0.05$) starting at day 7 post SPS to the same extent in day 21 and day 28 groups. To ascertain any effects on corticosterone (CORT) levels, serum CORT levels were measured by RIA. We observed a significant interaction between SPS and PRX-treatment on serum CORT levels at day 28 [$F(1, 15) = 16.72$, $p=0.001$], however, no significant interactions or differences were noted between groups in CORT levels at day 21. Increased serum N/OFQ in SPS rats ($p<0.05$) was abrogated in rats receiving PRX from day 0-21 of SPS ($p<0.05$). Additionally, N/OFQ levels

were measured regionally. For each brain region at each time point, a two-way ANOVA with Tukey's multiple post hoc comparisons were performed. There was a significant interaction between SPS stress and PRX treatment in PAG, hippocampus and cortex from day 28 rats: N/OFQ levels were elevated by SPS and reversed back to baseline with PRX treatment. No changes in N/OFQ levels in the amygdala were noted at either time point. Results from day 21 animals were very similar but not as pronounced, suggesting that N/OFQ levels were dynamically changing between days 21 and 28. These results suggest that PRX may exert its nociceptive effects, at least in part, via central modulation of N/OFQ.

Disclosures: P. Dib: None. **K.M. Standifer:** None. **Y. Zhang:** None. **C.D. Simpson-Durand:** None.

Nanosymposium

287. Mood Disorders: Novel Therapeutic Mechanisms

Location: 140A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 287.13

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Fear extinction facilitation by fluoxetine is mediated by basolateral amygdala endocannabinoids

Authors: *O. GUNDUZ CINAR[1], S. FLYNN[1], R. CINAR[1], T. S. RAMIKIE[2], G. KUNOS[1], S. PATEL[2], A. HOLMES[1];
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Abstract: Recent evidence implicates endocannabinoids in the protection and recovery from psychological stress. Previous work has shown that augmenting endocannabinoid anandamide, via inhibition of the catabolic enzyme fatty acid amide hydrolase (FAAH), facilitates fear extinction in mice. This effect is mediated via CB1 receptors in basolateral nucleus of the amygdala (BLA) and is associated with facilitation of synaptic plasticity in the BLA (long-term depression of inhibitory transmission). Interestingly, recent studies also demonstrate that chronic treatment with the selective serotonin reuptake inhibitor (SSRI) fluoxetine, a first-line therapeutic treatment for anxiety and stress-related neuropsychiatric conditions, facilitates fear extinction and promotes BLA plasticity in a similar manner to FAAH inhibitors. However, it's currently unclear whether fluoxetine's effects on fear extinction involve functional interactions with endocannabinoids. Here, we examined the contribution of BLA endocannabinoids to the extinction-facilitating effects of fluoxetine in mice. We first tested for changes in FAAH activity

and endocannabinoids in the BLA following chronic fluoxetine, and found a significant decrease in FAAH activity and a corresponding elevation in BLA anandamide (but not 2-AG) levels, as well as in the dorsal striatum and dorsal hippocampus. Next, we tested if combined treatment with fluoxetine and a FAAH inhibitor had additive effects on extinction, and whether the increased BLA endocannabinoid activity mediated fluoxetine's effects on extinction by blocking CB1 receptors. Pre-extinction-training systemic or bilateral intra-BLA CB1 receptor blockade occluded the extinction-facilitating effects of chronic fluoxetine. Using slice electrophysiological recordings, we showed that chronic fluoxetine significantly augmented CB1 receptor-dependent tonic suppression of inhibitory GABAergic transmission in the BLA, but did not alter 2-AG-mediated depolarization-induced suppression of inhibition (DSI). Collectively, these data suggest a scheme in which fluoxetine-induced increases in anandamide to release an inhibitory constraint on extinction-mediating neuronal activity in the BLA. Ongoing experiments seek to elucidate key molecular signaling pathways mediating fluoxetine's effects on the BLA endocannabinoid system, including calcineurin (protein phosphatase 2B). Taken together, these findings demonstrate a novel, obligatory role for amygdala endocannabinoids in the extinction-promoting effects of a major pharmacotherapy for anxiety disorders. This work was supported by NIAAA Intramural Research Program.

Disclosures: O. Gunduz Cinar: None. **S. Flynn:** None. **R. Cinar:** None. **T.S. Ramikie:** None. **G. Kunos:** None. **S. Patel:** None. **A. Holmes:** None.

Nanosymposium

288. Eye Movements and Perception

Location: 144A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 288.01

Topic: D.05. Visual Sensory-motor Processing

Support: Heart & Stroke Society, Canada

NSERC, Canada

Title: Remapping of multiple object features across eye movements

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Abstract: We constantly make eye movements yet our surroundings are perceived as being stable. Theoretically, spatial constancy requires that we accurately integrate information about objects across saccadic eye movements, comprising two components, 1) memorization of the object features and 2) updating of these features across eye movements, i.e. we need to remember where an object was and it's features at that location. However, not much is known about how we remap multiple features across eye movements. Here, we tested how accurately participants remembered multiple features of an object across saccadic eye movements. We asked seven subjects to compare two bars, each varying in location (1.6° left of center to 1.6°right in 0.4° intervals on the horizontal meridian), orientation (5° counterclockwise to 5° clockwise in 2° intervals) and size (1.8° to 2.3° in 0.1° increments). Both bars were viewed peripherally and sequentially with an intervening delay during which they either remained fixated or made a saccade to the opposite side. Participants reported how the second bar was different from the first for 1) all three attributes in each trial or 2) only one attribute within a block of trials. We found that remembering three attributes increased uncertainty about each attribute for all three features ($p<0.01$). Participants were most uncertain about the bar location following a saccade compared to when they remained fixated ($p<0.01$), mostly resulting from a bias in remembering the first bar to be closer to final fixation after the saccade. The intervening saccade also degraded the certainty of the orientation of the bar ($p<0.01$) and induced a reduction in the remembered size of the first bar ($p<0.05$). Based on the findings, we conclude that remapping of objects across saccades introduces uncertainty in their remembered attributes. When more attributes required memorization, uncertainty increased which points towards memory interactions between different visual features and saccadic eye movements.

Disclosures: **A.Z. Khan:** None. **Y. Nam:** None. **K. YoungWook:** None. **G. Blohm:** None.

Nanosymposium

288. Eye Movements and Perception

Location: 144A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 288.02

Topic: D.05. Visual Sensory-motor Processing

Support: NSERC Discovery Grant and the NSERC CANACT CREATE Program

Title: A state space model for trans-saccadic updating of remembered visual targets

Authors: *Y. MOHSENZADEH[1,2], D. CRAWFORD[1,2,3];
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Network and NSERC CAN-ACT CREATE Program, Toronto, ON, Canada; [3]Departments of Biology, Psychology, and Kinesiology & Hlth. Sciences, York Univ., Toronto, ON, Canada

Abstract: When we intend to make a saccade, our brain predicts an internal estimate of the future retinal target location. Therefore, when our eyes fall in the final post-saccadic position, the visual information is updated relative to the new gaze position; the process of trans-saccadic updating. This phenomenon has been the subject of many theoretical studies, but there still exists no general computational framework to explain this and related phenomenon. We propose a state space model for the updating of target-related spatial information in gaze-centered coordinates. The state space model is a probabilistic model which is used for analyzing dynamical systems. This model involves three kinds of signals: control input signals, noisy observed output signals and hidden states. In our proposed model for the updating mechanism, the control input is the eye velocity (efference copy) signal which starts before the saccade onset. The observed signal is the eye position which is observed during the saccade (this signal is noisy because of the error inherently existing in the sensory system). The hidden states are interpreted as the internal model of visual spatial information which is updated across the saccade. In our model, the hidden states are estimated based on a first order Markov process which models the memory for the updating. Our proposed state space model includes three sets of parameters: a state matrix which represents the transition between states, an input matrix which transforms the input control signal to the states space and also an output matrix which converts the internal states to the observations. We employ an Expectation Maximization (EM) approach during a training stage to estimate the model parameters. In the EM procedure, the log likelihood of the observed signal given the model parameters is maximized in two steps (which are known as E-step and M-step), recursively. Using the initial model parameters, the input and observed signals, the hidden states are estimated based on the Kalman filter in E-step. Then using the estimated hidden states in the previous E-step and also the input and observed signals, the parameters of the model are estimated and updated in the M-step and will be used in the next E-step as the new estimated parameters. These two steps are performed recursively to converge to a local optimum. Employing this model, we suggest that the updating of the hidden states is the mechanism underlying the trans-saccadic updating process. Our future plans are to 1) incorporate this framework within a more comprehensive model of trans-saccadic integration of general visual information, and 2) use this framework to construct a physiologically plausible model.

Disclosures: Y. Mohsenzadeh: None. **D. Crawford:** None.

Nanosymposium

288. Eye Movements and Perception

Location: 144A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 288.03

Topic: D.05. Visual Sensory-motor Processing

Title: A model of perisaccadic receptive field remapping in LIP predicts a moving wave of activity across the cortex

Authors: *M. ZHANG[1], S. WU[1], S. GUAN[1], C. FUNG[2], X. WANG[3], M. GOLDBERG[3]; [1]Beijing Normal Univ., Beijing, China; [2]Hong Kong Univ. of Sci. and Technol., Hong Kong, China; [3]Columbia Univ. Col. of Physicians and Surgeons, New York, NY

Abstract: Saccades induce shifts of visual images on the retina, yet we perceive a stable world. Predictive remapping of neuronal receptive fields (RFs) prior to a saccade is a potential strategy to compensate for these saccade-induced shifts. We showed (Wang et al, SfN 2008) that immediately before a saccade, a remapping neuron will respond to stimuli at spatial locations that are neither in the current RF (CRF) or future RF (FRF), when the saccade will sweep the retinal RF across those intermediate locations. We propose a computational model to decipher the underlying mechanism. For simplicity we present a one-dimensional version of the model. Consider a network with two classes of inputs: a set of visual inputs, and a set of corollary discharge inputs representing saccades of different amplitudes. We take advantage of the 'moving hill' of saccade activity in the superior colliculus: when a monkey makes a large amplitude saccade, activity begins in the intermediate layers of the caudal colliculus with neurons whose movement field describes the actual saccade. The activity migrates towards the rostral colliculus, so that saccadic burst cells with movement fields closer to the center of gaze start to discharge at times closer to the saccade, until the rostral colliculus begins to discharge when the saccade ends (Munoz et al.). To mimic the experimental protocol, we apply a visual stimulus in the future RF (FRF) of a visually responsive neuron. The stimulus activates a neuron whose RF lies in the spatial location of the FRF. This neuron is connected to an adjacent cortical neuron whose RF lies on a line between the FRF and the current RF (CRF) of the remapping neuron. Although the FRF neuron cannot ordinarily excite the adjacent neuron, the presence of the corollary discharge signal enables the FRF neuron to excite the neuron. The second neuron, in turn, projects to an adjacent neuron on the same line, which can now discharge because it is also excited by the corollary discharge of the impending saccade. The activity spreads along the cortex until the stimulus in the FRF can excite the cell whose RF is ordinarily limited to the CRF. Thus, the elongation of neuronal RF along the saccadic trajectory is achieved. This model has several advantages: it does not require that the entire retina projects to each neuron, nor does it require an inhibitory mechanism to prevent the neuron from responding ordinarily to a stimulus in the FRF. It makes the prediction that a cortical neuron whose RF lies between the CRF and the FRF but does not include either will briefly discharge when, during a remapping

process, the wave of cortical activity sweeps across its RF. We are currently testing this prediction in our laboratories.

Disclosures: **M. Zhang:** None. **S. Wu:** None. **S. Guan:** None. **C. Fung:** None. **X. Wang:** None. **M. Goldberg:** None.

Nanosymposium

288. Eye Movements and Perception

Location: 144A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 288.04

Topic: D.05. Visual Sensory-motor Processing

Support: NSERC PGSD2 459756-2014

NSERC RGPIN 341534-07

CIHR Mop-9222

Title: Gamma coherence accompanies receptive field remapping in monkey area V4

Authors: ***S. NEUPANE**, D. GUITTON, C. C. PACK;
Montreal Neurolog. Inst., Montreal, QC, Canada

Abstract: Predictive remapping of visual receptive fields (RFs) prior to execution of a visually guided saccade has been observed in multiple visual areas. During remapping, neurons transiently respond to stimuli flashed, prior to the eye movement, in their future RF location parallel to the impending saccade vector. Other findings suggest that RFs converge towards the saccade target (Tolias et. al., 2001, Zirnsak et. al., 2014) irrespective of the saccade vector. The mechanisms underlying remapping are unknown. One mechanism that could theoretically support remapping is a change in the coherence of gamma oscillations between distant sites on a retinotopic map. Gamma coherence has previously been shown to facilitate communication between brain areas, and we reasoned that it might similarly permit a transfer of stimulus information within a single region, as occurs during remapping. We tested this hypothesis by recording from multi-electrode arrays while monkeys performed a saccade task. We mapped visual receptive fields by flashing visual probes at random locations and times relative to the execution of saccades in different directions. We found that peri-saccadic RFs mapped with both spikes and LFPs showed receptive field shifts parallel to the saccade vector, consistent with standard accounts of remapping. In LFPs, such shifts were, in addition, accompanied by a

subsequent shift towards the saccade target. Peri-saccadic (spike) RFs of a minority of neurons showed a shift towards the saccade target (similar to Tolias et. al. 2001 and Zirnsak et. al. 2014), while another minority showed a mixture of the two types. Cells that showed a clear tendency to remap had spiking outputs that were more strongly locked to the gamma oscillations (40-60Hz) found in the LFPs. This suggested a potential role for gamma synchrony in visual remapping. Consistent with this hypothesis we found that gamma coherence increased around the time of saccades for electrodes whose RFs were separated by a distance equal to that of the saccade vector. Thus there was enhanced gamma coherence between neuronal pairs encoding the RF and future RF; we also found a similar increase in coherence between electrodes encoding the RF and those encoding the saccade target. We conclude that the sub-threshold neuronal activity reflected in LFP gamma coherence provides a mechanism for transiently updating representations of visual space around the time of each saccade.

Disclosures: **S. Neupane:** None. **D. Guitton:** None. **C.C. Pack:** None.

Nanosymposium

288. Eye Movements and Perception

Location: 144A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 288.05

Topic: D.05. Visual Sensory-motor Processing

Support: European Research Council (grant agreement 313568)

Title: Continuous perception: Interactions between saccadic remapping and temporal integration windows

Authors: *D. MELCHER[1,2], A. WUTZ[2], E. MUSCHTER[2], A. FRACASSO[3];
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[3]Exptl. Psychology, Utrecht Univ., Utrecht, Netherlands

Abstract: Perception of complex objects, scenes and events involves the integration of visual information across multiple time scales. For relatively low-level feature integration processes, as well as perceived simultaneity in vision and across the senses, numerous studies have reported integration windows of around 40 ms. Other visual integration phenomena, such as integration masking, operate on time scales of around 100 ms. Perception of apparent motion and several attentional phenomena have been shown to involve time periods of a few hundred milliseconds. Two stimuli separated by more than 500 ms may still interact but tend not to be viewed as a

single spatiotemporal object. An important question is how these different time windows interact to give the impression of continuous perception, particularly in situations in which a saccadic eye movement dramatically alters visual input. By studying behavioral measures of temporal integration around the time of eye movements, we have found that relative brief visual integration windows (< 100 ms) are disrupted peri-saccadically and effectively "reset" shortly after the onset of the new fixation. Medium temporal integration windows for object features, operating over the span of a few hundred milliseconds, continue across saccades and are consistent with an active, spatiotemporal remapping process based on attended objects. In particular, exact timing of temporal integration windows relative to eye movements might play a critical role for the impression of visual stability on rapid time scales. Such fast, feed-forward computations might still involve retinotopic coordinates and therefore require saccadic remapping. However, much of the impression of visual stability might involve longer time windows that are not entirely retinotopic and thus do not require saccadic remapping. Overall, this pattern of results suggest that visual stability emerges from the hierarchy of temporal integration windows, only some of which involve saccadic remapping, and, moreover, that many studies reporting spatial remapping can be re-interpreted in terms of time.

Disclosures: D. Melcher: None. **A. Wutz:** None. **E. Muschter:** None. **A. Fracasso:** None.

Nanosymposium

288. Eye Movements and Perception

Location: 144A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 288.06

Topic: D.05. Visual Sensory-motor Processing

Title: Remapping of touches on the skin during saccades

Authors: V. HARRAR[1,2], H. FRENZ[3], L. R. HARRIS[2], *M. LAPPE[3];
[1]Sch. of Optometry, Univ. of Montreal, Montreal, QC, Canada; [2]Psychology Dept., York Univ., Toronto, ON, Canada; [3]Inst. for Psychology, Muenster, Germany

Abstract: The remapping of visual receptive fields prior to a saccade has been demonstrated, behaviourally, by mislocalising visual stimuli flashed during a saccades. Here, we demonstrate the same pattern of errors for tactile stimuli. We tested the perceived location of a touch on the arm presented around the time of a saccadic eye movement. Subjects performed a visually-guided saccade from an LED located at the elbow to an LED on the forearm. One of four tactile stimulators arranged along the forearm tapped the participant at a random delay relative to the

saccade (including just before, during, or just after it). A second tap occurred at the same location, but 1.5 seconds later (after fixation had been re-established). Participants were asked to report the perceived relative position of the two touches. Touches presented just before the saccade were perceived as shifted to the right (the same direction as the saccade), while touches presented just after the saccade were perceived as shifted to the left, the same pattern of peri-saccadic shifts as found with visual stimuli. These results support the previous hypothesis that touch is coded in a visual reference frame, and further suggest that the position of tactile stimuli is quickly remapped with every eye movement. Remapping non-visual stimuli following eye movements suggests that multisensory receptive fields are kept in alignment across eye movements. Remapping appears to be critical for a stable perception of supramodal space, which itself appears to be visually guided. The intraparietal sulcus in the posterior parietal cortex seems the likely candidate for generating a multisensory spatial representation of the environment that responds predictively to eye-position.

Disclosures: **V. Harrar:** None. **H. Frenz:** None. **L.R. Harris:** None. **M. Lappe:** None.

Nanosymposium

288. Eye Movements and Perception

Location: 144A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 288.07

Topic: D.05. Visual Sensory-motor Processing

Support: CIHR MOP 9222

Title: Visual remapping is more impaired in patients with unilateral parietal lesion than in hemidecorticate patients as revealed by novel version of the double step task

Authors: *K. RATH-WILSON, D. GUITTON;
Montreal Neurolog. Inst., Montreal, QC, Canada

Abstract: Studies of remapping abilities in human patients with distinct cortical lesions are inconclusive. Patients with parietal lobe lesions, primarily of the right side, tested on the classical double-step task have a particular deficit in generating an ipsilesional saccade if it follows a contralesional saccade (Duhamel et al, 1992 & Heide et al, 1995). This deficit has been explained as an inability to generate / interpret corollary discharge for saccades elicited by the lesioned hemisphere. Recent studies, however, have called this finding into question. A review has re-interpreted the data from these earlier publications, suggesting that these results are

actually evidence of right-hemisphere dominance in human visual remapping (Pisella et al, 2011). Several studies of patients with right parietal lesions have determined that ipsilesional but not contralesional eye movements can result in a deficiency in remembering spatial information from previous fixations (Vuilleumier et al, 2007 & Russel et al, 2010). We tested hemidecorticate subjects on a novel version of the double-step task, adapted because our patients are hemianopic. We found that they do not have any impairment in remapping in either direction. We have tested a right parietal patient with this novel double-step task and found that he is unable to generate a contralesional saccade when it follows an ipsilesional saccade, in opposition to the findings of Duhamel et al, 1992. We are in the process of testing more parietal lesion patients with our novel paradigm to provide further insight into the saccadic remapping system in humans.

Disclosures: K. Rath-Wilson: None. **D. Guitton:** None.

Nanosymposium

288. Eye Movements and Perception

Location: 144A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 288.08

Topic: D.04. Vision

Support: NIH Grant EY18363

NSF Grant 1127216

NSF Grant 1420212

Title: Controlling attention at the microscopic scale

Authors: *M. POLETTI[1], M. RUCCI[2];
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Abstract: Visual attention is often studied by selectively attending to stimuli in the visual periphery. This is a natural approach as the control of attention is tightly linked to the planning of saccades, eye movements which relocate objects of interest from the periphery to the fovea. It is, however, unknown whether attention can be selectively allocated to stimuli separated by microscopic angular distances within the fovea. Recent studies have shown that humans direct saccades, with amplitude smaller than the foveola, toward nearby objects of interest (Ko et al, 2010), improving performance in high acuity tasks by compensating for non-uniform visual

acuity within the foveola (Poletti et al, 2013). On the basis of these findings, we speculated that as the visuomotor system is capable of controlling these microscopic gaze shifts, it should also be capable of exerting a comparable level of control over attention, selectively processing stimuli at different foveal locations. To test this prediction, we used a Posner task, a standard paradigm in which subjects report the appearance of targets at cued and uncued locations. While Posner tasks are commonly used with targets in the visual periphery, here we examined two conditions: the first one with targets presented at 3 deg from the center of gaze, and the second one with smaller targets (a 5 arcmin dot) presented at only 10 arcmin from the preferred locus of fixation. Testing attention at such small foveal eccentricities is challenging because fixational eye movements continually displace the retinal projection of the stimulus over an area as large as the foveola. To circumvent this issue, we stabilized stimuli on the observer's retina. That is, we updated the position of the objects on the display by means of a high precision eyetracker coupled with a gaze contingent display system to compensate for the observer's eye movements so to maintain the stimuli at a fixed foveal eccentricity. We show that the traditional Posner effect also holds within the foveola: reaction times were larger (by approximately 25 ms) in the detection of targets presented at non-attended locations, both at 3 deg and 10 arcmin eccentricity. Attentional shifts within the fovea were characterized by longer reaction times (43 ± 15 ms) than attentional shifts from the fovea to the periphery, a phenomenon similar to the longer latencies of microsaccades relative to saccades. These results indicate that we are capable of selectively attend to stimuli separated by as little as 10 arcmin, and further support the view that attention and eye movements are mediated by the same neural mechanism.

Disclosures: **M. Poletti:** None. **M. Rucci:** None.

Nanosymposium

288. Eye Movements and Perception

Location: 144A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 288.09

Topic: D.05. Visual Sensory-motor Processing

Support: EU FET SpaceCog

Title: Spatial compression: A consequence of relocalizing following disruption of the visual stream by masks or saccades

Authors: *P. CAVANAGH[1,2], S. BORN[1,2], E. ZIMMERMANN[3];
[1]Univ. Paris Descartes, Paris, France; [2]CNRS UMR 8242, Paris, France; [3]Inst. of Neurosci. and Med., Research Center Jülich, Germany

Abstract: Previous research has reported dramatic localization errors around the time of an eye movement. Stimuli briefly flashed just before a saccade are perceived closer to the saccade target, a phenomenon known as perisaccadic compression of space (Ross, Morrone, & Burr, 1997). We have demonstrated that similar mislocalizations of flashed stimuli can be observed in the absence of saccades (Zimmermann, Fink, & Cavanagh, 2013): Brief probes were attracted towards a visual reference when followed by a mask. We now examine the effect of color correspondence and find that both mask and saccade-induced compression are reduced similarly when the reference (or saccade target) and the probe have different rather than matching colors. We suggest that spatial compression is a consequence of the matching mechanism that attempt to relocalize targets following disruptions in the input, regardless of their origin.

Disclosures: P. Cavanagh: None. **S. Born:** None. **E. Zimmermann:** None.

Nanosymposium

288. Eye Movements and Perception

Location: 144A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 288.10

Topic: D.05. Visual Sensory-motor Processing

Support: BMBF Grant 01GQ1005C

GIF Grant 110879.1/2010

Title: The dynamics of transsaccadic attention shifts in area MT of the macaque

Authors: *T. YAO[1], S. TREUE[1,2,3], S. KRISHNA[1];
[1]Cognitive Neurosci. Lab., German Primate Ctr., Goettingen, Germany; [2]Bernstein Ctr. for Computat. Neurosci., Goettingen, Germany; [3]Fac. for Biol. and Psychology, Goettingen Univ., Goettingen, Germany

Abstract: Maintaining attention on relevant stimuli across eye movements is both critical and natural in our daily life. While recent psychophysical studies have shed light on the dynamics of attention shifts across saccades, we focused on the neuronal correlates of such transsaccadic attention shifts in the visual cortex of non-human primates. We recorded the extracellular activity

of single neurons in area MT of two macaque monkeys during a task that required them to make a saccade while maintaining attention on one of four moving random dot patterns (RDPs) in order to respond to a brief direction change in that target RDP by releasing a lever. In the first experiment, the target was either in the neurons' pre-saccadic receptive field (RF) or directly opposite to it, while in the second experiment, the target was either in the post-saccadic RF or directly opposite to it. By assessing the neuronal responses just before and after the saccade, we were able to determine the time course of attentional modulation of neuronal activity when the target was brought into or moved out of the neuron's RF by a saccade. Attentional enhancement of neuronal activity emerges within 60 ms after saccade offset when the attended stimulus enters the RF after the saccade. Comparing the results from the two experiments, we find that the locus of peak activity in MT switches back to the cued spatial location at the same time. The behavioral performance of the two monkeys indicates that the performance is impaired around the time of the saccade, but recovers within 50 ms after saccade offset in a manner consistent with the physiological data. Put together, our observations indicate a rapid repositioning of the top-down attentional locus in MT's retinotopic map across saccades.

Disclosures: **T. Yao:** None. **S. Treue:** None. **S. Krishna:** None.

Nanosymposium

288. Eye Movements and Perception

Location: 144A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 288.11

Topic: D.05. Visual Sensory-motor Processing

Support: Canadian Institutes of Health Research (CNS-90910)

National Science Foundation (BCS-0827764)

Defense Advanced Research Projects Agency (HR0011-10-C-0034)

Title: Visual coding in the superior colliculus during free-viewing of natural dynamic stimuli

Authors: *B. J. WHITE[1], D. BERG[3], L. ITTI[4], D. P. MUNOZ[2];
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Abstract: Much of what is known about the neural basis of vision and eye movements is through the use of simple stimuli in highly controlled laboratory settings. However, sensorimotor systems

evolved in natural environments where sensory information is complex and dynamic, and observers are free to act at their own volition. Thus, sensory coding may only be fully understood under such conditions. To this end, we examined visual saliency coding during free-viewing of natural dynamic stimuli. Saliency is thought to be governed by a distributed network of predominantly cortical brain areas, but there is growing evidence that the evolutionarily old midbrain superior colliculus (SC; optic tectum in non-mammals) plays a central role in this regard. The primate SC is a multilayered midbrain structure with visual representations in the superficial-layers (SCs), and sensorimotor representations linked to the control of eye movements/attention in the intermediate-layers (SCi). We tested the hypothesis that the SCs embodies the role of a visual saliency map, and the SCi embodies the role of a behavioral priority map (the combined representation of saliency and relevancy/value associated with a stimulus). We recorded single-units in the SC of two Rhesus monkeys during free-viewing of natural video. We used a computational model to estimate saliency at any retinal location, at any point in time. We sorted the total fixations (~80,000) into tertiles of averaged model-predicted saliency (low, med, high) in the response field (RF) around the time of fixation. The results showed a systematic increase in peri-fixation discharge with increasing levels of model-predicted saliency. We then examined a subset of the fixations based on the direction of the next saccade (IN vs ANTI the RF), under the assumption that saccade direction coarsely indicates the top-down goal of the animal (i.e., the value associated with the goal-directed stimulus). SCs neurons showed an enhanced response for greater model-predicted saliency irrespective of next-saccade direction, indicating that these neurons encode saliency irrespective of relevancy. In contrast, SCi neurons showed an enhanced response for greater saliency only when the stimulus that evoked it was the goal of the next-saccade (i.e., was of interest/value). This is interesting because SCs receives inputs exclusively from the retina and visual cortex, whereas SCi receives inputs predominantly from parietal and frontal cortices, which have been traditionally associated with saliency coding. The results support functionally distinct roles of SCs and SCi, whereby the former embodies the role of a visual saliency map, and the latter a priority map.

Disclosures: **B.J. White:** None. **D. Berg:** None. **L. Itti:** None. **D.P. Munoz:** None.

Nanosymposium

288. Eye Movements and Perception

Location: 144A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 288.12

Topic: D.06. Eye Movements

Support: NSERC 'Brain in Action' CREATE Program

Canada Research Chair Program

Title: Spatiotopic vs. retinotopic mechanisms of trans-saccadic feature integration: An fMRIa paradigm

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Abstract: The human visual system is able to retain and integrate a limited amount of visual feature information across saccades, but the neural mechanisms for this are little understood. A recent fMRIa study (Dunkley & Crawford SFN Abstracts 2013) found adaptation effects to stimulus orientation in a spatiotopic frame in bilateral parietal cortices, suggesting that these areas may play a role in the spatiotopic integration of object orientation images across saccades. Here, we used a similar fMRIa paradigm, but further investigated which cortical areas are involved in feature integration. In particular, the presentation of the stimuli was manipulated such that the stimuli appeared in novel or repeated orientation in retinotopic, spatiotopic and frame-independent conditions. Similar to Dunkley & Crawford (2013), 13 participants took part in an orientation-discrimination task while maintaining fixation on one of two possible fixation crosses. Within each trial, two circular stimuli comprised of obliquely oriented lines were presented sequentially one after the other. The lines could be presented in the same orientation (45° or 135°) in both stimulus presentations within each trial, or in different orientations (45° firstly and 135° secondly, or vice versa). There was a total of 24 trials in each block, and six blocks altogether. Participants were instructed to indicate via a button press whether the orientations of the stimuli within each set of two were the same (Repeated) or different (Novel). In our analysis, we investigated adaptation effects to stimulus orientation in each of the three spatial conditions. In particular, we expected adaptation (higher BOLD) for novel vs. repeated stimulus orientation. This analysis revealed significant adaptation effects to the orientation of the stimulus in the retinotopic condition in bilateral supplementary eye fields (SEF), left supramarginal (SMG) and left intraparietal sulcus. In the spatiotopic condition, we found significant adaptation within the left primary motor area (M1), left inferior parietal lobe (IPL) and left SMG. In the frame-independent condition, we found significant adaptation in right pre-SEF, left SPL and left IPL. These results corroborate the findings by Dunkley & Crawford (2013) that parietal cortex plays a role in low-level object feature processing, such as orientation. In addition, we find here that this occurs in a way stimulus orientation in a manner that is independent of the frame of reference. This suggests that trans-saccadic integration of stimulus

orientation in parietal cortices allows a stable representation of low-level object features independently of reference frame.

Disclosures: **B. Baltaretu:** None. **B.T. Dunkley:** None. **S. Monaco:** None. **J.D. Crawford:** None.

Nanosymposium

288. Eye Movements and Perception

Location: 144A

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 288.13

Topic: D.05. Visual Sensory-motor Processing

Support: NSF Graduate Research Fellowship

Title: Computational modeling evidence for a contribution of predictive eye position to the remapping of visual receptive fields

Authors: ***H. RAO**, F. SHEN, J. SANJUAN, K. RAFIE, J. VILLA, M. A. SOMMER;
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Abstract: Many neurons in the primate visual system exhibit presaccadic remapping: they shift their receptive fields prior to a saccade by combining visual and oculomotor information. We designed a computational model to test the hypothesis that presaccadic remapping is needed to achieve visual stability. We operationally defined “visual stability” as the accurate localization of visual objects despite eye movements. Our model received visual information from a moveable camera representing the eye, which fed into hierarchical, recursively connected neural network sheets, culminating in a layer representing the frontal eye field (FEF). This FEF layer received both visual information and corollary discharge -- a copy of the eye/camera movement command from a simulated superior colliculus. The entire system was tasked with one goal: to guide a robotic arm to visual targets despite saccadic movements of the camera. We found that through the course of training, neurons in the FEF sheet developed presaccadic remapping. It appeared that the purpose of the remapping was to counter predictive eye position signals. Specifically, the latency of the remapping matched those associated with the updating of internal representation of eye position. The result was a constantly accurate head-centered representation of visual objects for reaching. The direction of the remapping was parallel to the saccade vector as found originally by Duhamel et al. (1992) and most other studies in FEF. Our results suggest that presaccadic remapping is linked to predictive eye position signals, and that shifts in the receptive

field parallel to the saccade are to be expected in tasks when eye position information is important. Such shifts seem to provide an elegant solution to the problem of maintaining a constant craniotopic representation of objects. Shifts in other directions, e.g. toward the saccade target (Zirnsak et al. 2014), may occur when other factors are more influential, such as spatial visual attention.

Disclosures: **H. Rao:** None. **F. Shen:** None. **J. SanJuan:** None. **K. Rafie:** None. **J. Villa:** None. **M.A. Sommer:** None.

Nanosymposium

289. The Cells and Molecules of Touch, Itch, and Thermoreception

Location: 143A

Time: Monday, November 17, 2014, 8:00 AM - 11:00 AM

Presentation Number: 289.01

Topic: D.09. Tactile/Somatosensory

Support: T32 NS073548

NIH NS075760

NIH NS050758

NIH NS033730

NIH NS023725

Title: Optical stimulation of keratinocytes activates cutaneous nociceptors

Authors: ***K. M. BAUMBAUER**, J. J. DEBERRY, P. C. ADELMAN, R. H. MILLER, B. M. DAVIS, K. M. ALBERS, H. R. KOERBER;
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Abstract: Cutaneous primary sensory neurons are thought to be the first in a chain of cells that convert environmental stimuli into recognizable sensations of touch, heat, cold and pain. It has been proposed that nonneuronal cells, specifically keratinocytes, contribute to the initial transduction process. In order to test this hypothesis we developed a transgenic mouse model expressing channelrhodopsin-2 (ChR2) in keratinocytes by crossing mice Ai32 expressing ChR2 driven by the Rosa-26 promoter (ChR2-EYFP) with K14- keratin Cre mice. The EYFP tag allowed for visualization of cells containing ChR2. These mice exhibited robust expression of

ChR2-EYFP in basal keratinocytes and hair follicles of hairy skin and basal and suprabasal keratinocytes of glabrous skin. In addition intense staining was also found in Merkel Cells. Behavioral testing revealed that 10 applications of blue light (473nm; 39.7mW; 30s) per mouse elicited nocifensive responses in approximately 30% of the applications. This stimulation never evoked these responses in control groups (i.e. K14 Cre, Ai32 or WT). We next used an ex vivo skin/nerve/DRG/spinal cord preparation to record from individual cutaneous sensory neurons and characterize their sensitivity to mechanical and thermal stimuli. Fiber conduction velocities and the responses to natural stimuli were used to identify different functional classifications of cutaneous nociceptors. Next blue light (39.7mW; 5-10s) was applied to the cell's receptive field to determine whether keratinocyte activation elicited action potential (AP) firing in the nociceptive fiber. If no response was elicited, we next paired blue light stimulation and natural stimuli (mechanical or thermal) to determine if keratinocyte activation elicited subthreshold responses in the nociceptive fibers. We found that keratinocyte activation elicited either suprathreshold or subthreshold responses in most types of cutaneous nociceptors. These included both C-fibers (CMH, CMHC, CH, CM, CMC, but not in CC or CLTMR) and A-fiber nociceptors (A-HTMR). Interestingly keratinocytes evoked responses in both polymodal nociceptors (mechanical and thermal) as well as those responding to a single modality. For A-LTMR fibers only the SA1 population responded to blue light stimulation. These results show that light activation of epidermal keratinocytes alone produces APs in multiple types of cutaneous sensory neurons, demonstrating that, sensory transduction mechanisms also reside in skin keratinocytes.

Disclosures: K.M. Baumbauer: None. **J.J. Deberry:** None. **P.C. Adelman:** None. **R.H. Miller:** None. **B.M. Davis:** None. **K.M. Albers:** None. **H.R. Koerber:** None.

Nanosymposium

289. The Cells and Molecules of Touch, Itch, and Thermoreception

Location: 143A

Time: Monday, November 17, 2014, 8:00 AM - 11:00 AM

Presentation Number: 289.02

Topic: D.09. Tactile/Somatosensory

Support: NIG Grant DA031777 (G.S.)

NIH Grant DA029204 (A.I.B.)

FAER Research Fellowship Grant (V.L.T.)

HHMI Medical Research Fellowship (S.A.L.)

Title: Control of spinal somatosensory and motor neural circuits by delta opioid receptors

Authors: V. L. TAWFIK[1], D. WANG[1], S. A. LOW[1], B. L. KIEFFER[3], A. I. BASBAUM[4], *G. SCHERRER[2,1];
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Abstract: Opioid receptors are G protein-coupled receptors that regulate neurotransmission throughout the nervous system, including along somatosensory neural circuits. Here we used mouse genetics, in situ hybridization, immunohistochemistry and electrophysiology to investigate the functional organization of opioid receptors in the spinal cord. We found that the delta opioid receptor (DOR) is expressed by numerous cell populations throughout the spinal grey matter. DOR+ cells express NeuN, but not glia markers Iba1, CD11b, P2Y12 and GFAP, indicating that they are neurons. In the dorsal horn DOR+ neurons are concentrated in lamina II inner. Coimmunolabeling revealed that the majority of these DOR+ neurons are calbindin+, a marker of excitatory interneurons, and a subset coexpresses PKCgamma. Whole-cell patch clamp recordings in slices showed that DOR+ neurons in lamina II inner displayed either single, delayed or gap action potential firing patterns, consistent with their excitatory nature. We next used dorsal root stimulation and found that these DOR+ neurons receive Adelta or Abeta afferent inputs, which likely correspond to myelinated low-threshold mechanoreceptors. We further show that postsynaptic activation of DOR opens G protein-coupled inwardly rectifying K+ (GIRK) channels, hyperpolarizing the membrane and reducing excitability to myelinated low-threshold mechanoreceptor inputs. We also report striking dorso-ventral segregation in the identity of DOR+ neurons. Thus in contrast with DOR+ neurons in the superficial dorsal horn, the majority of DOR+ neurons located in lamina III and more ventral coexpress markers of inhibitory neurons. Specifically, DOR is expressed by populations of NOS+ inhibitory interneurons in lamina III and by parvalbumin+ inhibitory neurons in lamina IV-V. Genetic labeling of neurons expressing the transcription factor engrailed 1 revealed that virtually all ventral horn V1 inhibitory interneurons express DOR. Consistent with this finding, triple immunolabeling indicated that DOR+ neurons in the ventral horn include calbindin+ Renshaw cells. Finally, we found that cerebellum-projecting Clarke's column neurons also express DOR. Collectively these results suggest that beside their action on primary afferent mechanoreceptors, DOR agonists could also decrease nerve injury-induced mechanical hypersensitivity by a postsynaptic action on lamina II excitatory interneurons. Furthermore, DOR expression in ventral horn inhibitory interneurons, including Renshaw cells, indicates a complex function for DOR in the modulation of both somatosensation and motor control in the spinal cord.

Disclosures: V.L. Tawfik: None. **D. Wang:** None. **S.A. Low:** None. **B.L. Kieffer:** None. **A.I. Basbaum:** None. **G. Scherrer:** None.

Nanosymposium

289. The Cells and Molecules of Touch, Itch, and Thermoreception

Location: 143A

Time: Monday, November 17, 2014, 8:00 AM - 11:00 AM

Presentation Number: 289.03

Topic: D.09. Tactile/Somatosensory

Title: Characterization of action potential discharge in chloroquine (CQ) and histamine-sensitive "itch nerves" terminating in mouse skin

Authors: F. RU[1], A. HERBSTOMER[1], S. MEEKER[1], *X. DONG[2], B. J. UNDEM[1];
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Abstract: CQ selectively activates somatosensory nerves via stimulation of MrgprA3 receptors. Knocking out MrgprA3 receptors or the subset of nerves that express MrgprA3 strongly inhibits CQ-induced itch (Cell. 2009,139:1353; Nat Neurosci. 2013,16:174). We therefore defined those nerves in mouse skin that are sensitive to activation by CQ as "itch nerves" and have characterized these nerves in a modified ex-vivo skin-dorsal root ganglion preparation. The thoraco-dorsal skin (with intact spinal nerves and DRGs T7-T10) was pinned epidermis-side down and superfused with Kreb's buffer (33oC). Dye spreading revealed that the cornium surface presents a diffusion barrier slowing the rate of drug access to nerve endings in the intradermal compartments. Therefore, we isolated the skin with the thoracodorsal artery intact. Applying capsaicin intrarterially vs. superfusion allowed for nerve activation at 10-fold lower concentrations with >10-fold quicker onset of action. CQ stimulated only C-fibers (CV=0.7 ± 0.02 m/s). Among 166 mechanosensitive C-fibers evaluated (generally 1-3 per animal), 28% were CQ-sensitive. Virtually all CQ-sensitive fibers were also histamine sensitive, and all CQ-insensitive fibers were also unresponsive to histamine. About 75% of the CQ-sensitive C-fibers were capsaicin sensitive. The peak discharge in these nerves averaged 12±2 Hz and 15 ± 2 Hz for CQ and capsaicin-respectively. Mustard oil (100 μM) a concentration that strongly activates vagal airway C-fibers, did not stimulate itch fibers, and at 300 μM evoked only 3 ± 1Hz (n=7). No quantitative differences were noted in the CQ-induced action potential discharge in cutaneous C-fibers of wild-type vs.TRPA1 -/- animals (peak frequency discharges of 12 ± 2 Hz vs. 13 ± 2 Hz, respectively). Moreover, scratching in response to intradermal injection of CQ was the same between wild-type and TRPA1-/- mice, averaging 263 ± 36 (n=15) vs. 238 ± 26 (n=19) bouts of scratching, respectively. Mustard oil failed to stimulate calcium responses in DRG neurons, or action potential discharge in airway C-fibers of the TRPA1 -/- mice. These data indicate that histamine and CQ activate the same population of itch C-fibers in mouse skin, and that TRPA1 is

not required for the transduction of MrgprA3 receptor activation to action potential discharge in mouse C-fiber itch terminals.

Disclosures: **F. Ru:** None. **A. Herbstomer:** None. **S. Meeker:** None. **X. Dong:** None. **B.J. Undem:** None.

Nanosymposium

289. The Cells and Molecules of Touch, Itch, and Thermoreception

Location: 143A

Time: Monday, November 17, 2014, 8:00 AM - 11:00 AM

Presentation Number: 289.04

Topic: D.09. Tactile/Somatosensory

Support: NIH Grant AR056318-01 (Z. F. C)

Title: The BNP-NPRA pathway functions independent of the GRP-GRPR pathway for itch sensation

Authors: ***D. M. BARRY**, X.-Y. LIU, L. WAN, F.-Q. HUO, H. LI, Z.-Q. ZHAO, Z.-F. CHEN; Anesthesiol., Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: A recent study identified BNP as an important peptide for itch transmission. However, the study claimed that BNP, but not GRP, is a major neurotransmitter for itch in pruriceptors, and BNP in dorsal root ganglion (DRG) neurons and its receptor NPRA in the spinal cord are a key signaling pathway for transmitting itch from the skin to the spinal cord. The study showed that NPRA is perfectly co-expressed with Grp mRNA in the spinal cord, and BNP-NPRA acts upstream of GRP-GRPR to relay itch. Our studies, along with the others, do not support their claims. We find that Grp mRNA in DRG is upregulated in chronic itch mice using both in situ hybridization and quantitative RT-PCR. GRP was detected in DRG cell bodies and spinal cord afferent fibers by immunostaining which was not observed in Grp KO. The majority of GRP spinal cord fiber staining is lost after dorsal rhizotomy. We find that scratching behaviors induced by BNP and GRP exhibited marked differences in the time-course, suggesting that the two peptides are unlikely to function in a cascade. We confirm that BNP is important for inhibiting inflammatory pain and morphine analgesia. Pharmacological and genetic blockade of GRP-GRPR signaling does not significantly affect i.t. BNP-induced scratching behavior. Accumulating evidence demonstrates that GRP is a key neurotransmitter for mediating histamine-independent itch in pruriceptors. Our findings demonstrate that BNP does not act

upstream of GRP. The function of BNP in itch and pain and its relationship with GRP remain to be further investigated.

Disclosures: **D.M. Barry:** None. **X. Liu:** None. **L. Wan:** None. **F. Huo:** None. **H. Li:** None. **Z. Zhao:** None. **Z. Chen:** None.

Nanosymposium

289. The Cells and Molecules of Touch, Itch, and Thermoreception

Location: 143A

Time: Monday, November 17, 2014, 8:00 AM - 11:00 AM

Presentation Number: 289.05

Topic: D.09. Tactile/Somatosensory

Title: Cold inhibits acute itch mediated by multiple pruritic pathways

Authors: *R. PALKAR[1], E. K. LIPPOLDT[2], D. D. MCKEMY[3];
[1]Neurosci. Grad. Program, [2]Neurobio. Grad. Program, Dept. of Biol. Sci., [3]Dept. of Biol. Sciences, Neurobio. Section, USC, Los Angeles, CA

Abstract: Itch (Pruritus) management poses an important clinical challenge due to the limited therapeutic options available for those affected with pathological itch. The source of this problem lies in the range of itch-inducing agents, the multitude of distinct itch receptors and divergent transduction pathways distributed among a range of sensory neurons that are involved in itch syndromes. This diversity hinders therapeutic interventions to prevent itch via receptor antagonism, making a “one-size fits all” approach unlikely. However, counter-stimuli such as scratching or cooling are known to relieve itch of most etiologies, representing a general antipruritic pathway. Cold has been used to treat itch for centuries, yet we know little of the underlying cellular and molecular mechanisms, likely due to the paucity of studies in this arena. Moreover, the efficacy of cold in treating various types of itch has not been well tested. Here, we examined the effects of moderately cool (17^0C) and painfully cold (10^0C) temperatures on mouse models of acute itch mediated by five pruritogens with distinct molecular mechanisms of itch-transduction, including histamine, chloroquine, Bam8-22, SLIGRL-NH2, and αMe-5HT. We find that moderate cooling (20^0C -17^0C) was able to inhibit some itch pathways, whereas inhibition of itch induced by other pruritogens required temperatures considered painfully cold (10^0C). Remarkably, the effects of cooling on itch were transient, and itch-behaviors re-emerged once the cold stimulus was removed. These results suggest that cold can be successfully used to treat multiple pathways of itch, if its effects can be prolonged. Future studies into the cellular and

molecular basis of cold-mediated itch inhibition will lead to novel insights into itch signaling, and perhaps show that using counter-stimuli as a means to block itch will be broadly applicable to all itch modalities.

Disclosures: **R. Palkar:** None. **E.K. Lippoldt:** None. **D.D. McKemy:** None.

Nanosymposium

289. The Cells and Molecules of Touch, Itch, and Thermoreception

Location: 143A

Time: Monday, November 17, 2014, 8:00 AM - 11:00 AM

Presentation Number: 289.06

Topic: D.08. Pain

Support: NIH IRP 1ZIAHD008884-04

Title: Heat responses in larval zebrafish: escape, thermotaxis and arousal

Authors: ***T. YOKOGAWA**[1], M. IADAROLA[2], H. A. BURGESS[1];
[1]NICHD, Bethesda, MD; [2]Dept. of Perioperative Med., NIH, Bethesda, MD

Abstract: Detection of the changing thermal environment is critical for animal survival. Sensory detection drives motor behaviors which allow animals to rapidly escape from noxious temperatures into an optimal thermal environment. Here we report three types of behavioral response to changes of the thermal environment. Responses of freely swimming zebrafish were monitored under three conditions: to a short thermal pulse with infra-red laser, to a temperature gradient across the testing arena, and to a slow increase in ambient water temperature. Larvae responded rapidly to the acute high intensity thermal pulse, and kinematic analysis demonstrated that they performed a swim pattern similar to a Mauthner cell mediated escape response. In contrast, larvae in a temperature gradient, showed thermotaxis, turning and swimming away from the region of high temperature. The third response, thermal arousal, was manifest as a short term change in internal state. When ambient water temperature was increased from baseline 26 C to a noxious 36 C, larvae became hyperactive. Importantly, hyperactivity persisted for several minutes after the water temperature returned to 26 C. During thermal arousal, fish showed normal movement initiation frequency but increased velocity during swim bouts. We speculate that thermal arousal prepares fish to anticipate and quickly respond to subsequent changes in environmental temperature. To investigate the neuronal basis for these different responses, we generated fish for optogenetic activation of trigeminal neurons and tracing of axonal projections. For this, we used an enhancer trap line which expresses Gal4 in a subset of trigeminal neurons.

Ablation of trigeminal neurons using UAS:nitroreductase resulted in a reduction of thermal responses confirming the involvement of the neurons in these behaviors. After injection of a UAS:channelrhodopsin2 plasmid, we activated trigeminal neurons in freely swimming fish. Individual fish stochastically expressed ChR2 in only a small subset of trigeminal neurons. ChR2 expressing fish initiated either escape responses or thermal arousal after trigeminal activation, demonstrating that distinct trigeminal neurons mediate these behaviors. We then traced axons from trigeminal neurons with defined behavioral roles revealing distinct projection patterns. These studies show that individual trigeminal neurons selectively drive different thermal behaviors.

Disclosures: T. Yokogawa: None. **M. Iadarola:** None. **H.A. Burgess:** None.

Nanosymposium

289. The Cells and Molecules of Touch, Itch, and Thermoreception

Location: 143A

Time: Monday, November 17, 2014, 8:00 AM - 11:00 AM

Presentation Number: 289.07

Topic: D.08. Pain

Support: NICHD T32

NIH NRSA Predoctoral F31

NIMH 2R15MH086928

NINDS R01NS086082

4-VA Consortium grant

NINDS R011NS069828

Title: Dissection of genetic and molecular bases of noxious cold detection using *Drosophila* larvae

Authors: *H. N. TURNER[1], K. ARMENGOL[2], S. IYER[2], L. SULLIVAN[2], E. R. IYER[2], C. LANDRY[3], D. N. COX[2], M. J. GALKO[1];
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Abstract: An organism's comfort and survival depends on its ability to detect and avoid noxious thermal stimuli capable of causing tissue damage. Although much is understood about noxious heat avoidance, our understanding of the basic biology of noxious cold perception is gravely minimal. Our goal is to determine the genetic basis for noxious cold perception using the genetically tractable Drosophila model. We developed novel tools for assessing cold nociception in Drosophila larvae including a "cold probe" that allows focal application of a defined noxious cold stimulus (3-15 ºC), as well as a global cold plate assay. In both assays Drosophila larvae produce a mutually exclusive set of primary reactive behaviors, distinct from the commonly reported aversive "corkscrew" behavior seen in response to a high temperature probe. These behaviors include a tail raise (TR), a combined head and tail raise (HT), and a full-body scrunching (SC) behavior. These behaviors are cold-specific, occurring in 60% of larvae below 12º C in the cold probe assay, and in over 80% of larvae below 8º C in the cold plate assay. Silencing synaptic transmission in Class III peripheral sensory neurons resulted in a significant loss in cold-evoked SC behavior in both assays. Additionally, when Class III neurons are stimulated by cold perfusion we see an increase in activity via GCaMP fluorescence. Finally, when Class III neurons are activated optogenetically we observe SC behavior. Increases in GCaMP ratios or optogentically-induced behavior were also observed to a lesser extent in Class II neurons in the global assay. Changes in GCaMP ratio, or optogentically-induced behavior were completely absent upon stimulating Class I or IV peripheral sensory neurons. Class III neurons express a number of Transient Receptor Potential (TRP) channels including TRPM, nompC, and almost there (Pkd2). Larvae mutant for these TRP channels showed a decrease in SC behavior in the cold probe and cold plate assays compared to controls. In addition, larvae with Class III sensory neuron-specific knockdown of these TRP channels using RNAi transgenes showed significant decreases in cold-evoked SC behavior in both assays. Together, these results suggest that the TRPM, nompC, and pkd2 TRP channels act in Class III neurons to detect and respond to noxious cold temperatures. These findings are particularly interesting since TRPM and Pkd2 channels are conserved and TRPM has been implicated in cold detection in vertebrates. We have established the first system to study noxious cold responses in Drosophila. Our unique tools and assays should allow us to further uncover the conserved molecular/genetic bases of noxious cold perception.

Disclosures: H.N. Turner: None. **S. Iyer:** None. **L. Sullivan:** None. **K. Armengol:** None. **E.R. Iyer:** None. **C. Landry:** None. **D.N. Cox:** None. **M.J. Galko:** None.

Nanosymposium

289. The Cells and Molecules of Touch, Itch, and Thermoreception

Location: 143A

Time: Monday, November 17, 2014, 8:00 AM - 11:00 AM

Presentation Number: 289.08

Topic: D.09. Tactile/Somatosensory

Support: HHMI

NIH

Title: A TRPV channel is required in recurved bristles on the wing margin of *Drosophila* to mediate its repulsive behaviors in response to mechanical stimulation

Authors: *W. ZHANG, J. LI, L. Y. JAN, Y. JAN;
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Abstract: The Drosophila body surface is covered by sensory bristles with different morphology. However, their functions are mostly unknown. By electrophysiological recording of the wing bristles, we found the recurved bristles on the wing margins are mechanosensitive to displacements. The responses depend on the strength of the mechanical stimulation and are directional selective. We then identified a TRPV-channel-specific Gal4 labels one neuron under each recurved bristle. Ablation of these neurons eliminates the displacement-triggered neuronal activities of the recurved bristles. Further, we found the TRPV channel is required for the mechanotransduction in the recurved bristles because the loss of function mutant lacks the mechanosensitivity. Moreover, we discovered that the flies exhibit repulsive behavior when the recurved bristles are gently stroked with a probe. This behavior requires the normal function of the TPRV channel in the neurons innervating the recurved bristles.

Disclosures: W. Zhang: None. **J. Li:** None. **L.Y. Jan:** None. **Y. Jan:** None.

Nanosymposium

289. The Cells and Molecules of Touch, Itch, and Thermoreception

Location: 143A

Time: Monday, November 17, 2014, 8:00 AM - 11:00 AM

Presentation Number: 289.09

Topic: D.09. Tactile/Somatosensory

Support: AFOSR grant FA95501210109

Title: Bats have evolved unique sensorimotor circuitry to support mammalian flight

Authors: *K. L. MARSHALL[1], M. CHADHA[4], L. A. DESOUZA[2], S. STERBING-D'ANGELO[5], C. F. MOSS[6], E. A. LUMPKIN[3];
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Abstract: Bats accomplish agile flight using modified hand-wings, with elongated digits connected by thin, flexible skin. Wing skin is endowed with microscopic hairs that modulate flight, which is proposed to occur through tactile receptors that provide feedback about airflow. Sensorimotor circuits develop through coordinated extension of motor and sensory innervation into surrounding tissue. The ordered projection of sensory neurons is the origin of topographical maps in CNS regions, which are organized somatotopically. In bats, somatotopic maps are atypical, displaying discontinuous representations of body areas and expanded forelimbs. We sought to investigate the peripheral basis of these unusual maps. The receptors and circuitry mediating sensorimotor integration in mammalian flight, and their developmental origins, are unknown. We study Eptesicus fuscus because they are fast-flying insectivores that perform acrobatic maneuvers mid-air to catch prey. We used neuronal tracing to identify sensory neurons and motor pools that innervate Eptesicus fuscus wings. The bat wing develops not only from the forelimb bud, but also the trunk and hindlimb bud. Thus, we hypothesized that unique sensorimotor circuits develop as a result of the wing's unusual ontogeny. Neuronal tracing experiments demonstrated that wing-skin sensory innervation arises from cervical (C) dorsal root ganglia (DRG) that innervate other mammalian forelimbs (C4-C8), as well as from mid- and lower thoracic (T) DRGs (T1-T9). By contrast, motor pools were located at cervical and upper thoracic spinal levels. Thus, spinal levels are mismatched between sensory and motor innervation. These data reveal the peripheral basis for the bat's unusual forelimb maps in somatosensory brain regions. Together, these findings indicate that bats have unique patterns of peripheral innervation in the wing. To analyze the structure and function of wing touch receptors, we used immunohistochemistry of wing skin to identify somatosensory end organs and in vivo recordings from primary somatosensory cortex to analyze firing in cortical neurons that receive inputs from wing somatosensory receptors. We found a novel repertoire of somatosensory receptors in wing skin. Analysis of cortical responses to touch and airflow indicated these stimuli are encoded through rapid signaling, which is essential to guide wing kinematics. Our findings reveal that evolutionary pressures enabling powered flight in bats have also given rise to somatosensory specializations of its hand-wing.

Disclosures: K.L. Marshall: None. **M. Chadha:** None. **L.A. deSouza:** None. **S. Sterbing-D'Angelo:** None. **C.F. Moss:** None. **E.A. Lumpkin:** None.

Nanosymposium

289. The Cells and Molecules of Touch, Itch, and Thermoreception

Location: 143A

Time: Monday, November 17, 2014, 8:00 AM - 11:00 AM

Presentation Number: 289.10

Topic: D.09. Tactile/Somatosensory

Support: VR

HFSP

Title: Edge-orientation processing in first-order tactile neurons

Authors: *A. PRUSZYNSKI, R. S. JOHANSSON;
IMB Physiology, Umea Univ., Umea, Sweden

Abstract: A fundamental feature of first-order neurons in the tactile system is that their distal axon branches in the skin and forms many transduction sites, yielding complex receptive fields with many highly sensitive zones. The functional consequences of this arrangement are unknown. Here, we will describe our recent work showing that this arrangement likely constitutes a peripheral neural mechanism for extracting the geometric features of touched objects. Specifically, we will show that two types of tactile afferent neurons (SA-1's and FA-1's) that densely innervate the glabrous skin of the human fingertips robustly signal edge orientation via both the intensity and the detailed temporal structure of their responses. Moreover, we will show that a neuron's sensitivity to edge orientation can be readily predicted from the spatial layout of its highly sensitive zones. Taken together, our findings emphasize that peripheral neurons in the touch-processing pathway, like peripheral neurons in the visual-processing pathway, can perform feature extraction computations traditionally attributed to neurons in the cerebral cortex.

Disclosures: A. Pruszynski: None. **R.S. Johansson:** None.

Nanosymposium

289. The Cells and Molecules of Touch, Itch, and Thermoreception

Location: 143A

Time: Monday, November 17, 2014, 8:00 AM - 11:00 AM

Presentation Number: 289.11

Topic: D.09. Tactile/Somatosensory

Support: NSF Grant IOS-1150209

Title: What the hand tells the brain: Predicting the responses of every cutaneous afferent in the hand to arbitrary spatio-temporal stimuli

Authors: *S. J. BENSMAIA, B. RAYHAUN, H. P. SAAL;
Dept. of Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL

Abstract: During object manipulation and tactile exploration, the human fingertip undergoes complex mechanical deformations. Coarse features, such as edges and corners, excite mainly slowly adapting type I and rapidly adapting fibers, which are densely packed in the fingertip. At the same time, interactions with objects and surfaces elicit high-frequency low-amplitude surface waves that propagate across the skin of the finger and palm, exciting vibration-sensitive Pacinian afferents all over the hand. Here, we present a comprehensive model that simulates the spiking responses of all the tactile afferents (i.e., across the entire hand) that are activated by mechanical stimulation of the fingertip. The model takes into account skin biomechanics and receptor biophysics and was developed based on the measured responses of afferents that innervate the glabrous skin of primates. The model is validated by showing that it can predict both the firing rate and the temporal patterning observed in the responses of individual mechanoreceptive afferents with high accuracy. We then use the model to predict perceptual judgments and find that psychophysical predictions derived from the simulated responses are as good as those derived from measured responses, further demonstrating that the model captures the aspects of the neural response that drive perception. The model will be invaluable for providing biomimetic somatosensory feedback in neuroprosthetic devices by allowing the generation of realistic peripheral response patterns from touch sensor outputs. It will also be an important tool in tactile research by allowing the fast and accurate simulation of the response evoked in the entire population of cutaneous mechanoreceptive afferents by any tactile stimulus.

Disclosures: S.J. Bensmaia: None. **B. Rayhaun:** None. **H.P. Saal:** None.

Nanosymposium

289. The Cells and Molecules of Touch, Itch, and Thermoreception

Location: 143A

Time: Monday, November 17, 2014, 8:00 AM - 11:00 AM

Presentation Number: 289.12

Topic: D.08. Pain

Support: NIH Grant DE023730

Scan Design Foundation Innovative Pain Research Grant

Title: Modeling pain discrimination in zebrafish: A way forward for unbiased analgesic discovery

Authors: A. CURTRIGHT, S. GOH, M. ROSSER, B. KEOWN, J. SHARIFI, E. WAGNER, *A. K. DHAKA;
Biol. Structure, The Univ. of Washington, Seattle, WA

Abstract: Despite massive investment, there has been limited success in the development of novel analgesic compounds. The dominant drug development model follows a predictable preclinical path in which a selected target is subjugated to high-throughput in vitro screens, the generation of lead compounds, and finally testing in disease model systems for safety and efficacy assessments. While this approach can be successful, it often leads to failure due to poor target selection, the inability to model complex pain behaviors using in vitro testing and/or ineffectiveness and unforeseen side effects in animal model testing. In fact many current drugs such as NSAIDS, gabapentin and ketamine were identified due to their analgesic properties prior to the identification of their targets. It would therefore be advantageous to develop high-throughput assays to model complex pain behaviors in which one could screen for novel analgesics that could modulate nociception at any of the many levels within the pain processing circuitry in an unbiased manner. While much work has been performed using rodents to study nociceptive behavior, screening for small molecule analgesics using these models is not practical due to the high cost, time and manpower necessary to conduct such a screen. The zebrafish provides an intriguing model system to study nociception. The neural circuits underling nociception in zebrafish larvae are highly analogous to those found in higher vertebrates such as rodents and humans. Furthermore we've shown that zebrafish larvae have a functionally diverse peripheral nervous system and respond robustly to noxious stimuli and their small size allows for rapid upscaling using existing high throughput platforms. Here we report the development of high-throughput discrimination assays that likely requires supraspinal processing to model acute and sensitized nociception with large numbers of zebrafish larvae. Furthermore, we show that these assays can be used to screen for small molecule analgesics, by showing that previously described analgesics with different pharmacological properties are able to suppress one or both noxious avoidance behaviors.

Disclosures: A. Curtright: None. **S. Goh:** None. **M. Rosser:** None. **B. Keown:** None. **J. Sharifi:** None. **E. Wagner:** None. **A.K. Dhaka:** None.

Nanosymposium

290. Neural Mechanisms of Energy Balance Regulation and Obesity

Location: 147B

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 290.01

Topic: E.07. Food Intake and Energy Balance

Support: NIH Grant NS048476

NIH Grant DK084052

Title: Neurophysiological properties of hypothalamic glutamate neurons in the perifornical area

Authors: D. J. SPERGEL, A. ZAYACHKIVSKY, *A. N. VAN DEN POL;
Dept Neurosurg., Yale Univ. Sch. Med., NEW HAVEN, CT

Abstract: Neurons in the perifornical region of the hypothalamus play roles in food intake, energy homeostasis, sleep and cognitive arousal, and addiction. Here we focused on excitatory neurons that utilize glutamate as a fast amino acid transmitter. To identify excitatory neurons we used a transgenic mouse that expresses GFP driven by the promoter for the vesicular glutamate transporter vGluT2. Neurons that contain hypocretin (Hcrt)/orexin are found in the same region of the hypothalamus, and may also employ glutamate and express vGluT2. Excitatory neurons showed a set of physiological and cytochemical signatures that differentiated them from Hcrt neurons, and this was corroborated by the absence of immunostaining for Hcrt using a rabbit anti-Hcrt antibody. To investigate the differences between the two cell types, we recorded and studied membrane properties of vGluT2 neurons in cell-attached and whole-cell modes. Hcrt neurons were identified in an Hcrt-GFP transgenic mouse. Similar to nearby inhibitory melanin-concentrating hormone neurons, only a small proportion of vGluT2 neurons were spontaneously active (22%, n = 32). In contrast, most Hcrt neurons showed spontaneous action potentials. Resting membrane potential in vGluT2 neurons (-69.4 ± 1.7 mV, n = 23) was more hyperpolarized than in Hcrt neurons (-64.3 ± 1.7 mV, n = 25). About half of the vGluT2 neurons recorded exhibited an after-depolarization following an action potential (48%, n = 23); this was rare in Hcrt neurons (4%, n = 25). vGluT2 neurons showed a greater degree of spike frequency adaptation than Hcrt neurons showed. The differences in the membrane properties of vGluT2 neurons compared to those of Hcrt neurons imply different functions or circuitry, and that vGluT2 neurons play roles that are parallel or additional to those of Hcrt neurons.

Disclosures: D.J. Spergel: None. **A.N. van den Pol:** None. **A. Zayachkivsky:** None.

Nanosymposium

290. Neural Mechanisms of Energy Balance Regulation and Obesity

Location: 147B

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 290.02

Topic: E.07. Food Intake and Energy Balance

Support: Penn University Research Foundation

Title: Blockade of AMPA/kainate receptors in the central nucleus of the amygdala reduces cisplatin-induced malaise likely through direct hindbrain projections

Authors: A. L. ALHADEFF[1], R. A. HOLLAND[2], *B. C. DE JONGHE[2];
[1]Psychology, [2]Biobehavioral Hlth. Sci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Nausea, vomiting, and anorexia are indicators of malaise that are associated with cisplatin chemotherapy in humans and animal models; however, the neural circuits mediating these effects are not fully understood. Our previous studies demonstrate that various hindbrain nuclei [e.g. nucleus tractus solitarius (NTS), area postrema, lateral parabrachial nucleus (lPBN)] as well as the central nucleus of the amygdala (CeA) are activated by systemic cisplatin treatment. Furthermore, recent literature implicates glutamatergic output from the NTS and lPBN in the cessation of feeding through circuits that mediate malaise. Given that there are direct projections from the NTS and lPBN to the CeA, we hypothesized that glutamatergic signaling in the CeA mediates cisplatin-induced malaise via direct NTS and lPBN projections. To test this hypothesis we (1) gave bilateral microinjections of CNQX, an AMPA/kainate glutamate receptor antagonist, to the CeA immediately before systemic cisplatin treatment and (2) performed double immunohistochemistry (IHC) in the NTS and lPBN for CeA-injected Fluorogold (monosynaptic retrograde tracer) and cisplatin-induced cFos immunoreactivity in rats. AMPA/kainate receptor blockade significantly attenuated cisplatin-induced pica, a well-established rodent model of nausea, but had no effect on cisplatin-induced anorexia. Furthermore, preliminary IHC data suggest that 20-25% of cisplatin-activated neurons in the rostral NTS, and 50-60% of cisplatin-activated neurons in the lPBN, project directly to the ipsilateral CeA. Planned studies will investigate the role of CeA NMDA glutamate receptor signaling in cisplatin-induced nausea and anorexia, and the neurochemical phenotypes of NTS and lPBN cisplatin-activated neurons. Together, these data begin to establish a glutamatergic circuit by which cisplatin chemotherapy causes malaise.

Disclosures: **A.L. Alhadeff:** None. **B.C. De Jonghe:** None. **R.A. Holland:** None.

Nanosymposium

290. Neural Mechanisms of Energy Balance Regulation and Obesity

Location: 147B

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 290.03

Topic: E.07. Food Intake and Energy Balance

Title: Diet composition is more important than caloric intake or body weight in remodeling the electrical properties of AgRP/NPY neurons in the arcuate nucleus of the hypothalamus

Authors: K. PHAM, A. SMITH, *K. O'CONNELL;
Physiol., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Obesity is a chronic condition resulting from a long-term pattern of poor diet and lifestyle choices. We recently showed that chronic consumption of a high-fat diet (HFD) leads to increased body weight and a persistent, inappropriate activation of orexigenic AgRP/NPY in the arcuate nucleus of the hypothalamus (ARH). However, the development and relative contributions of diet composition and body weight that contribute to these changes remain unclear. To investigate the impact of short-term HFD consumption on AgRP/NPY neuronal excitability, mice were fed *ad libitum* HFD for 48 h and spontaneous firing rates from these neurons in acute brain slices were then measured. Even short-term HFD consumption – which does not result in a measurable increase in body weight – lead to a significant increase in AgRP neuronal spiking to a rate nearly identical to chronically HFD-fed mice. Since even brief exposure to HFD results in a decrease in leptin-dependent JAK2/STAT3 signaling in AgRP /NPY neurons, we determined if the same was true for neuronal firing. Remarkably, we found that 100 nM leptin still induced a significant decrease in spike frequency in short-term HFD-fed mice (aCSF : 3.0 0.9 Hz; Leptin: 0.6 0.2 Hz, n=4), suggesting that leptin-dependent regulation of spike rate is distinct from the JAK2/STAT3 pathway. Mice fed HFD will initially overfeed, though eventually their caloric intake will match standard diet (SD)-fed controls. To determine the role of diet composition (HFD vs. SD) in the absence of differences in calorie consumption, we next fed mice a calorie restricted high-fat diet (HFD-CR) corresponding to the average daily intake of the SD-fed group (~12 kcal/mouse/day). Remarkably, even on a restricted calorie diet these mice gained significant weight compared to the SD-fed group, although they gained less weight than mice fed HFD *ad libitium*. AgRP neurons in brain slices from HFD-CR mice exhibited increased spiking nearly identical to mice fed *ad libitum*, suggesting that diet composition may be more important than calorie content in electrically remodeling arcuate AgRP/NPY neurons.

Disclosures: K. Pham: None. **K. O'Connell:** None. **A. Smith:** None.

Nanosymposium

290. Neural Mechanisms of Energy Balance Regulation and Obesity

Location: 147B

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 290.04

Topic: E.07. Food Intake and Energy Balance

Support: R01 DK079194

Title: Maternal high fat diet consumption decreases dopamine signaling in prefrontal cortex of non-human primate (NHP) juvenile offspring

Authors: *H. M. RIVERA, E. L. SULLIVAN, P. KIEVIT, M. A. KIRIGITI, L. BAUMAN, S. R. LINDSLEY, K. BAQUERO, P. BLUNDELL, T. A. DEAN, M. S. SMITH, K. L. GROVE; Div. of Diabetes, Obesity, and Metabolism, Oregon Natl. Primate Res. Ctr., Beaverton, OR

Abstract: Using a NHP model, our laboratory has shown that maternal high-fat diet (HFD) consumption during gestation and lactation is associated with metabolic-related complications in offspring. More recently, we have shown that HFD offspring have an increased preference for a diet high in fat and sugar. These animals appear to have reduced food reward signals and compensate by overeating highly palatable diets. The dopamine (DA) system is widely known to regulate food reward, therefore, we hypothesized that maternal HFD consumption decreases central DA signaling in offspring. In the present study, we targeted the prefrontal cortex (PFC) due to the involvement of this region in higher-order executive functions, such as food reward. Our studies show that maternal HFD consumption causes a decrease in DA fiber innervation specifically in the superficial layer of the PFC, as indicated by a decrease in the number of tyrosine hydroxylase expressing fibers. Furthermore, there was a decrease in the dopamine receptor 2 mRNA levels in the pyramidal cell layer of the PFC. Together, our findings reveal that maternal HFD consumption decreases DA signaling in the PFC, indicating a likely disruption in the food reward pathway.

Disclosures: H.M. Rivera: None. **E.L. Sullivan:** None. **P. Kievit:** None. **M.A. Kirigiti:** None. **L. Bauman:** None. **S.R. Lindsley:** None. **K. Baquero:** None. **P. Blundell:** None. **T.A. Dean:** None. **M.S. Smith:** None. **K.L. Grove:** None.

Nanosymposium

290. Neural Mechanisms of Energy Balance Regulation and Obesity

Location: 147B

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 290.05

Topic: E.07. Food Intake and Energy Balance

Title: Serotonergic neurons mediate hunger in adult *Drosophila*

Authors: *S. D. ALBIN[1], K. R. KAUN[2], J.-M. KNAPP[1], P. M. CHUNG[1], U. HEBERLEIN[1], J. H. SIMPSON[1];
[1]Janelia Farm, ASHBURN, VA; [2]Dept. of Neurosci., Brown Univ., Providence, RI

Abstract: Motivational drive states strongly influence a variety of complex behaviors. For example, hunger affects food seeking and feeding behaviors, as well as appetitive memory acquisition and performance. In adult Drosophila, neurons involved in taste processing, motor control of feeding, and metabolism have been identified, but none of these alter motivational drive states such as to mimic hunger. To identify missing components of the "hunger circuit," we performed a behavioral screen for neurons whose acute activation can mimic hunger in a fed fly. We identified a small population (~50 neurons) of primarily serotonergic central brain neurons. Activating these neurons in a sated fly using a temperature sensitive cation channel led to starvation-related behaviors such as increased ingestion, increased proboscis extension to sucrose, and increased preference for nutritive sugars over non-nutritive sugars. Inhibiting these neurons in starved flies decreased, but did not eliminate, feeding. In addition, the expression of appetitive memory in flies depends on motivational state and is promoted by hunger. We found that activating this subset of neurons in fed flies specifically during memory retrieval mimics the effect of hunger on memory. Conversely, activating these neurons in starved flies during memory acquisition blocked appetitive memory formation, suggesting that reduced hunger drive is a salient cue for memory acquisition. Importantly, activating all serotonergic neurons in the fly does not mimic hunger, suggesting a specific local action of these serotonergic neurons in coordinating the hunger-induced behaviors. The sparsity of this serotonin pattern will facilitate future research into the physiology and connectivity of these neurons. This work provides insights into how neural circuits encode internal state and how changes in the internal state lead to broad coordinated effects on complex behaviors.

Disclosures: S.D. Albin: None. **J. Knapp:** None. **P.M. Chung:** None. **U. Heberlein:** None. **K.R. Kaun:** None. **J.H. Simpson:** None.

Nanosymposium

290. Neural Mechanisms of Energy Balance Regulation and Obesity

Location: 147B

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 290.06

Topic: E.07. Food Intake and Energy Balance

Support: NIH Grant F31NS082027

NIH Grant T32GM008322

Start-up funds (University of Michigan, Department of Pediatrics)

Title: Nos1 neurons of the paraventricular nucleus of the hypothalamus control feeding and energy expenditure

Authors: *A. K. SUTTON[1,2], H. PEI[3], K. H. BURNETT[3], C. J. RHODES[4], M. G. MYERS JR.[2], D. P. OLSON[3];
[2]Mol. and Integrative Physiol., [3]Dept. of Pediatrics, [1]Univ. of Michigan, Ann Arbor, MI; [4]Dept. of Intrnl. Med., Univ. of Chicago, Chicago, IL

Abstract: The paraventricular nucleus of the hypothalamus (PVH) contains a heterogeneous cluster of Sim1-expressing cell types that form the major autonomic output nucleus from the hypothalamus. PVH neurons play critical roles in the control of food intake and energy homeostasis; however, the various roles of specific PVH neuronal subtypes in energy balance have yet to be defined. Nitric oxide synthase-1 (Nos1) is expressed in a subset of Sim1-expressing PVH ($Sim1^{PVH}$) neurons but their function in energy balance is unknown. To determine the role of $Nos1^{PVH}$ neurons in feeding and energy expenditure, we employed Cre-dependent viral vectors to both map efferent projections and test their functional output. We show that $Nos1^{PVH}$ neurons project to hindbrain and spinal cord regions important for food intake and energy expenditure control, respectively. Moreover, pharmacogenetic activation of $Nos1^{PVH}$ neurons suppresses feeding to a similar extent as $Sim1^{PVH}$ neurons and increases energy expenditure and locomotor activity. Furthermore, we found that oxytocin-expressing PVH neurons (OXT^{PVH}) are a subset of $Nos1^{PVH}$ neurons. OXT^{PVH} cells project to pre-ganglionic, sympathetic neurons in the thoracic spinal cord and increase energy expenditure upon activation. We are currently assessing if this increase in energy expenditure via OXT^{PVH} neuronal activation is driven by UCP1-induced thermogenesis in brown adipose tissue. Somewhat surprisingly, activation of OXT^{PVH} neurons fails to alter feeding. Thus, $Nos1^{PVH}$ neurons promote negative energy balance through changes in feeding, energy expenditure and activity, whereas OXT^{PVH} neurons regulate energy expenditure alone. This suggests a crucial role for non-OXT $Nos1^{PVH}$ neurons in the central regulation of energy balance.

Disclosures: **A.K. Sutton:** None. **H. Pei:** None. **K.H. Burnett:** None. **C.J. Rhodes:** None. **M.G. Myers Jr.:** None. **D.P. Olson:** None.

Nanosymposium

290. Neural Mechanisms of Energy Balance Regulation and Obesity

Location: 147B

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 290.07

Topic: E.07. Food Intake and Energy Balance

Support: NIG Grant MH093650

NIG Grant MH091945

NIG Grant DA030425

Title: PACAP within the central nucleus of the amygdala reduces food intake via the melanocortin and the BDNF/TrkB systems

Authors: *A. FERRAGUD FAUS[1], A. IEMOLO[2], P. COTTONE[2], V. SABINO[2];
[2]Pharmacol. and Psychiatry, [1]Boston Univ., Boston, MA

Abstract: The pituitary adenylate cyclase-activating peptide (PACAP) and its receptor PAC1 are present in high concentrations not only in the hypothalamus but also in extrahypothalamic regions such as the central nucleus of the amygdala (CeA). PACAP, administered intracerebroventricularly and into the hypothalamus, reduces food intake and body weight gain. The present study sought to investigate whether the PACAP/PAC1 of the CeA plays a role in the regulation of food intake and body weight in rats, and its mechanism of action. Bilateral administration of PACAP (0-1 μg/rat) into the CeA of ad libitum fed male, adult Wistar rats significantly reduced food intake and body weight gain in a dose-dependent manner. Microstructure analysis of feeding revealed that rats injected with PACAP in the CeA ate smaller meals of normal duration. This reflected that PACAP slowed down feeding within meals. PACAP treatment did not influence postprandial satiety, meal frequency or inter-meal intervals. Microinfusion of PACAP into the CeA did not affect locomotor activity, ruling out malaise or a general suppression on behavior. Importantly, the anorectic effect of PACAP was prevented by the selective melanocortin MCR3/MCR4 antagonist SHU9119, also microinfused into the CeA. In addition, intra-CeA microinfusion of the Trk receptor inhibitor k252a, also blocked the anorectic effect of PACAP. In summary, our data prove that PACAP signaling within the CeA

has profound effects on food intake and reveal a mechanism of action involving the local POMC/MCR4 and the BDNF/TrkB systems in the CeA, downstream targets of the PAC1 receptor.

Disclosures: **A. Ferragud Faus:** None. **A. Iemolo:** None. **P. Cottone:** None. **V. Sabino:** None.

Nanosymposium

290. Neural Mechanisms of Energy Balance Regulation and Obesity

Location: 147B

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 290.08

Topic: E.07. Food Intake and Energy Balance

Support: NIH grant MH081817

Title: Maternal high-fat diet (HFD) increases anxiety-like behavior and reduces the number of oxytocin (OT)-positive neurons in the paraventricular nucleus of the hypothalamus (PVN) in adult male offspring

Authors: S. KOJIMA[1], *L. M. RINAMAN[2];
[1]Dept. of Neurosci., [2]Univ. Pittsburgh, Pittsburgh, PA

Abstract: Maternal HFD persistently increases body weight and anxiety-like behavior in adult male offspring (Bilbo & Tsang, 2010). Since central OT signaling reduces anxiety, we hypothesized that central OT systems are downregulated in the offspring of maternal HFD rats. To test this hypothesis, timed-pregnant Sprague-Dawley rats were given ad lib access to either standard chow (CHOW: Purina 5001) or HFD (60% kcal fat; Research Diets D12492) from gestation day 2/3, continuing through parturition [offspring postnatal day (P)0] and lactation. On P1 litter size was reduced to 8-10 pups of both sexes. On P21, all pups were weaned to standard chow and housed together with same-sex siblings. At 9 wks of age, male and female offspring were tested on the elevated plus-maze (EPMZ). Two weeks later, offspring were injected i.p. with saline or bacterial lipopolysaccharide (LPS; 100 μg/kg BW), then perfused with fixative 2.5-3 hr later. Tissue sections were processed for immunoperoxidase localization of cFos-positive (i.e., activated) neurons, followed by immunofluorescence localization of OT. In the EPMZ test, male HFD offspring made fewer entries into the open arms and spent less time there compared to CHOW male offspring, whereas female offspring displayed no maternal diet-related differences. Compared to male CHOW offspring, male HFD offspring displayed significantly fewer OT-positive neurons within the PVN. Further, the number of OT-positive PVN neurons in

male offspring was positively correlated with the ratio of open:closed arm entries, such that increased anxiety-like EPMZ behavior was associated with fewer OT-positive neurons, and vice-versa. Compared to saline injection, LPS significantly and similarly increased PVN OT neuronal activation in male offspring of both maternal diet groups. Compared to CHOW offspring, HFD offspring displayed a non-significant trend towards increased OT neural activation after i.p. saline. We conclude that maternal HFD reduces the number of OT-positive PVN neurons in male offspring, which may contribute to their increased anxiety-like behavior.

Disclosures: S. Kojima: None. **L.M. Rinaman:** None.

Nanosymposium

290. Neural Mechanisms of Energy Balance Regulation and Obesity

Location: 147B

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 290.09

Topic: E.07. Food Intake and Energy Balance

Support: FAU EFI Neurotrition

Title: Molecular determinants of the palatability of snack food and their effects on whole brain activity patterns

Authors: T. HOCH[1], S. KREITZ[2], M. PISCHETSRIEDER[1], *A. HESS[3];
[1]Dept. of Chem. and Pharm., Food Chem. Unit, Erlangen, Germany; [2]Inst. of Pharmacol. & Toxicology, Erlangen, Germany; [3]I.F. Pharmacol & Toxicol, Erlangen, Germany

Abstract: Snack food like potato chips substantially contributes to energy intake in humans. In contrast to basic food, snacks are consumed additionally to other meals and may thereby lead to non-homeostatic energy intake and hyperphagia. Our aim was to identify the active molecular compounds of potato chips, which are possible triggers for overruling the homeostatic energy balance and to elucidate their influence on whole brain activity patterns. For this purpose, we performed two-choice preference tests with rats, presenting test foods with the content of fat, carbohydrates or the mixture of fat and carbohydrates as existing in potato chips. We found that the fat or carbohydrates alone led to an enhanced intake compared to standard chow. The presentation of potato chips led to the highest intake but the mixture of both main nutrients of potato chips - fat and carbohydrates - induced an intake comparable to potato chips. Thus, we concluded that the mixture of fat and carbohydrates is a major molecular determinant of potato chips triggering hedonic hyperphagia. Next we investigated the impact of the intake of crushed

potato chips, the maximal attractive mixture of fat and carbohydrates and standard chow on the feeding behavior and whole brain activity patterns. The integral activity of 166 brain areas during 7 days of access to the respective test foods accompanied by the release of the contrast agent manganese chloride from osmotic pumps was measured by manganese-enhanced MRI (MEMRI: imaging sequence MDEFT, TR 4 s, TE 5.2 ms, TI 1000 ms, matrix 256 x 256 x 64, FOV 27.90 x 27.90 x 28.16 mm with a resolution of 109 x 109 x 440 µm, 2 averages). Significant differences became obvious concerning the animal behavior and activation of distinct brain structures dependent on the ingested food. Rats with access to potato chips showed the highest feeding related locomotor activity, followed by the mixture of fat carbohydrates and standard chow. Main differences in the brain activities between rats, which had access to potato chips, and rats with access to standard chow could be detected in brain areas involved in circuits regulating reward and addiction, food intake, sleep and locomotor activity. These differences were qualitatively and quantitatively more pronounced when potato chips were consumed compared to the mixture of fat and carbohydrates. In conclusion, potato chips strongly influence the feeding related locomotor activity and the activity of several brain areas like structures of the reward system. Despite of the highly attractive ratio of the components fat and carbohydrates, this seems not to be the only trigger.

Disclosures: **T. Hoch:** None. **S. Kreitz:** None. **M. Pischetsrieder:** None. **A. Hess:** None.

Nanosymposium

290. Neural Mechanisms of Energy Balance Regulation and Obesity

Location: 147B

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 290.10

Topic: E.07. Food Intake and Energy Balance

Support: NIH NIDDK grant R01DK063592

NIH NIDDK grant R01DK099722

AHA Grant-in-Aid 13GRNT16120004

UCSF Diabetes Family Fund

NIH NIDDK grant K01DK098320

AHA Postdoctoral Fellowship 12POST10690005

NIH NIMH grant R01MH081880

Title: Estrogen-responsive Tac1 neurons in the ventrolateral region of the ventromedial hypothalamus (VMHvl) selectively regulate physical activity and body weight in female mice

Authors: *S. CORREA[1], D. W. NEWSTROM[1], J. P. WARNE[2], P. FLANDIN[3], C. C. CHEUNG[1], A. T. LIN-MOORE[1], A. A. PIERCE[4], A. W. XU[2], J. L. RUBENSTEIN[3], H. A. INGRAHAM[1];
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Abstract: Central estrogen signaling via estrogen receptor alpha (ERα) regulates reproduction, food intake, basal thermogenesis, and physical activity in female mice. Whereas ERα is required in both the arcuate (ARC) and ventromedial (VMH) hypothalamic nuclei for fertility, ERα regulates food intake and basal thermogenesis via the ARC and the VMH, respectively. However, the ERα neurons controlling physical activity remain undefined. Here we employ complementary loss- and gain-of-function strategies to target ERα$^+$ VMH neurons. We conditionally ablated *Nkx2-1* (*NK2 homeobox 1* or *Ttf-1*), a transcription factor that is expressed in hypothalamic progenitors and upregulated in adult VMH_{VL} neurons, using *Sf1*-driven Cre recombinase (*Nkx2-1*^{Sf1Cre}). Female *Nkx2-1*^{Sf1Cre} mice are 30% heavier than littermate controls when fed normal chow, with increased visceral and subcutaneous adiposity. Male body weight is unaffected. Metabolic analyses revealed that obesity is due to reduced energy expenditure rather than increased food intake; *Nkx2-1*^{Sf1Cre} females exhibit a specific deficit in physical activity. In the *Nkx2-1*^{Sf1Cre} VMH, we observe a selective decrease in neurons co-expressing NKX2-1, ERα and *Tachykinin 1* (*Tac1*). *Tac1* is enriched in VMH_{VL} neurons and expressed higher in females. Despite decreased ERα, female *Nkx2-1* ^{Sf1Cre} mice are fertile, a finding that uncouples the roles of hypothalamic ERα in reproduction and metabolism. To demonstrate that *Nkx2-1*$^+$ VMH_{VL} neurons regulate locomotion, Cre-dependent Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) were used to activate *Nkx2-1Cre*$^+$ VMH_{VL} neurons. Activation of *Nkx2-1*$^+$ VMH_{VL} neurons results in a burst of physical activity that is more pronounced in females than in males and is modulated by ovarian hormones. Furthermore, the increase in locomotion is blunted in ERα- or *Tac1*-deficient mice, suggesting functional roles for these female-biased VMH_{VL} markers in regulating physical activity. Our findings demonstrate that estrogen-responsive *Tac1*$^+$ VMH_{VL} neurons constitute an important part of a previously undefined locomotor circuit that maintains energy homeostasis in females.

Disclosures: S. Correa: None. **D.W. Newstrom:** None. **J.P. Warne:** None. **P. Flandin:** None. **C.C. Cheung:** None. **A.T. Lin-Moore:** None. **A.A. Pierce:** None. **A.W. Xu:** None. **J.L. Rubenstein:** None. **H.A. Ingraham:** None.

Nanosymposium

290. Neural Mechanisms of Energy Balance Regulation and Obesity

Location: 147B

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 290.11

Topic: E.07. Food Intake and Energy Balance

Support: NIH Grant F32 DK089710

NIH Grant R01 DK096010

NIH Grant R01 DK089044

NIH Grant R01 DK071051

NIH Grant R01 DK075632

NIH Grant R37 DK053477

Title: Deciphering the wiring diagram of the agrp neuronal circuit

Authors: *M. J. KRASHES[1], B. SHAH[2], D. OLSON[3], N. UCHIDA[4], B. LOWELL[2]; [1]NIH, Bethesda, MD; [2]Beth Israel Deaconess Med. Ctr., Boston, MA; [3]Univ. of Michigan, Ann Arbor, MI; [4]Harvard Med. Sch., Boston, MA

Abstract: Hunger is a hard-wired motivational state essential for survival. Agouti-related peptide (AgRP)-expressing neurons in the arcuate nucleus (ARC) at the base of the hypothalamus are crucial to the control of hunger. They are activated by caloric deficiency and, when naturally or artificially stimulated, they potently induce intense hunger and subsequent food intake. Consistent with their obligatory role in regulating appetite, genetic ablation or chemogenetic inhibition of AgRP neurons decreases feeding. Excitatory and inhibitory input to AgRP neurons is important in caloric-deficiency-induced activation, and is notable for its remarkable degree of caloric-state- dependent synaptic plasticity. Despite the important role of excitatory and inhibitory input, its source(s) has been unknown. Here, through the use of Cre-recombinase-enabled, cell-specific neuron mapping techniques in mice, we have discovered strong excitatory drive that, unexpectedly, emanates from the hypothalamic paraventricular nucleus, specifically from subsets of neurons expressing thyrotropin-releasing hormone (TRH) and pituitary adenylate cyclase-activating polypeptide (PACAP, also known as ADCYAP1). Chemogenetic stimulation of these afferent neurons in sated mice markedly activates AgRP neurons and induces intense feeding. Conversely, acute inhibition in mice with caloric-deficiency-induced hunger decreases feeding. For GABAergic inputs to AgRP neurons, we have

identified potent inhibitory drive originating in the dorsal medial hypothalamus, specifically from subsets of neurons expressing leptin receptor. Optogenetic stimulation of these ARC-projecting LepRDMH neurons silences AgRP activity and results in aphagia, a condition that is reversed upon lifting this inhibitory tone. Discovery of these afferent neurons capable of triggering hunger alterations advances understanding of how this intense motivational state is regulated.

Disclosures: **M.J. Krashes:** None. **B. Shah:** None. **B. Lowell:** None. **N. Uchida:** None. **D. Olson:** None.

Nanosymposium

290. Neural Mechanisms of Energy Balance Regulation and Obesity

Location: 147B

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 290.12

Topic: E.07. Food Intake and Energy Balance

Support: PhD grant CORDDIM 2012

Societe Francophone du Diabete

University Paris Diderot

Title: Olfactory-bulbar prokineticin-2 is involved in the regulation of food intake and glucose homeostasis

Authors: M. MORTREUX, N. KASSIS, S. MIGRENNE-LI, *C. MAGNAN;
Univ. Paris Diderot, Paris, France

Abstract: The energy balance is finely regulated by the central nervous system (CNS), wherein the olfactory bulb (OB) participates in the initiation of food intake. In humans and animals, the olfactory sensitivity is increased by fasting and decreased by satiation1. Obese patients have a lower threshold for odor detection2 that might explain, at least in part, a deregulation of their feeding behavior. Prokineticin-2 (Prok2) is a secreted protein widely expressed in the CNS and especially in the OB, where it plays a role in the establishment of projections towards the hypothalamus3. Patients with the Kallmann Syndrome caused by a mutation of the PROK2 gene or its receptor display anosmia, hypogonadotrophic-hypogonadism and in some cases a severe obesity4. In rodents it was recently evidenced that Prok2 decreases food intake via a hypothalamic pathway5. The aim of our work was to assess the role of olfacto-bulbar Prok2 in

the regulation of food intake and glucose homeostasis. We first evidenced by qPCR that Prok2 mRNA level in the OB of C57Bl/6j mice is decreased after 24h fasting in lean mice under chow diet (CD), whereas it is increased in high fat diet-induced-obese mice, compared to the fed state. This suggests that Prok2 mRNA expression in the OB depends on both the nutritional (fed/fasted) and the metabolic (lean/obese) status of the animal. In order to test whether changes in Prok2 expression in OB may impact food intake either recombinant Prok2 or shRNA against Prok2 was injected in OB of both mice fed CD or HFD. Acute stereotaxic injection of recombinant Prok2 significantly decreased food intake 24h post injection compared to vehicle infused mice, whatever the metabolic state. A contrario, effects of a chronic down-expression of Prok2 in the OB using a lentiviral vector expressing a shRNA against Prok2 induced an increase in food intake and altered glucose homeostasis in both lean and diet-induced-obese mice.Our data show that olfacto-bulbar Prok2 is involved in the control of food intake and glucose homeostasis and that its expression is altered in obese mice, suggesting that this protein could be a new potential target for the treatment of metabolic diseases such as obesity and type 2 diabetes. To go further in the action mechanisms of olfacto-bulbar Prok2, we started to study its expression in leptin-deficient ob/ob mice, whose hyperphagic behavior is linked to an altered olfactory sensitivity. 1Aimé, P. et al. (2007) Behavioural Brain Research 2Richardson, B. E et al. (2004) Obesity Surgery 3 Negri, L., et al. (2007) Life Sciences 4Dodé, C. and P. Rondard (2013) Frontiers in endocrinology 5Gardiner, J. V., et al. (2010) Diabetes

Disclosures: **M. Mortreux:** None. **C. Magnan:** None. **N. Kassis:** None. **S. Migrenne-Li:** None.

Nanosymposium

290. Neural Mechanisms of Energy Balance Regulation and Obesity

Location: 147B

Time: Monday, November 17, 2014, 8:00 AM - 11:15 AM

Presentation Number: 290.13

Topic: E.07. Food Intake and Energy Balance

Support: NIH Grant DK31405

NIH Grant DK90861

DFG (German Research Foundation)

K99 (K99NS087096)

Title: Exercise induces hippocampal BDNF through a PGC-1alpha/FNDC5 pathway

Authors: *C. D. WRANN[1,2], J. P. WHITE[1,2], J. SALOGIANNIS[3], D. MA[4], J. D. LIN[4], M. E. GREENBERG[3], B. M. SPIEGELMAN[1,2];
[1]Dana-Farber Cancer Inst., Boston, MA; [3]Neurobio., [2]Harvard Med. Sch., Boston, MA; [4]Life Sci. Inst. and Dept. of Cell and Developmental Biol., Ann Arbor, MI

Abstract: Exercise can improve cognitive function and has been linked to the increased expression of brain-derived neurotrophic factor (BDNF). However, the underlying molecular mechanisms driving the elevation of this neurotrophin remain unknown. Here we show that FNDC5, a previously identified muscle protein that is induced in exercise and is cleaved and secreted as irisin, is also elevated by endurance exercise in the hippocampus of mice. Neuronal Fndc5 gene expression is regulated by PGC-1a and Pgc1a-/- mice show reduced Fndc5 expression in the brain. Forced expression of FNDC5 in primary cortical neurons increases Bdnf expression, whereas RNAi-mediated knockdown of FNDC5 reduces Bdnf. Importantly, peripheral delivery of FNDC5 to the liver via adenoviral vectors, resulting in elevated blood irisin, induces expression of Bdnf and other neuroprotective genes in the hippocampus. In addition, stimulation of primary hippocampal neurons with recombinant irisin activates a similar gene program. Taken together, our findings link endurance exercise and the important metabolic mediators, PGC-1a and FNDC5, with BDNF expression in the brain. We are currently investigating the identity of the irisin receptor.

Disclosures: C.D. Wrann: None. **J.P. White:** None. **J. Salogiannis:** None. **D. Ma:** None. **J.D. Lin:** None. **M.E. Greenberg:** None. **B.M. Spiegelman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ember Therapeutics. F. Consulting Fees (e.g., advisory boards); Ember Therapeutics.

Nanosymposium

291. Sleep and Memory

Location: 206

Time: Monday, November 17, 2014, 8:00 AM - 10:45 AM

Presentation Number: 291.01

Topic: E.08. Biological Rhythms and Sleep

Title: Identification of a single nucleotide substitution specific to the Sleepy mutant mouse pedigree by linkage analysis and whole exome sequencing

Authors: *H. FUNATO[1,2], C. MIYOSHI[2], M. SATO[3], A. IKKYU[2], N. HOTTA[2], M. KAKIZAKI[2], S. KANNO[2], K. HARANO[2], F. ASANO[2], T. FUJIYAMA[2], T. SUZUKI[4], S. WAKANA[4], M. YANAGISAWA[2,3,5];
[1]Dept. of Anat., Toho Univ. Sch. of Med., Tokyo, Japan; [2]Univ. Tsukuba, WPI-IIIS, Tsukuba, Japan; [3]Univ. Texas Southwestern Med. Ctr., Dallas, TX; [4]RIKEN BRC, Tsukuba, Japan; [5]HHMI, Dallas, TX

Abstract: Although sleep is a ubiquitous animal behavior, the molecular mechanism of sleep homeostasis remains unknown. We performed high-throughput screening of ENU-mutagenized mice in order to identify genes regulating sleep/wake behavior. We have so far analyzed EEG/EMG data of more than 6,000 mutagenized male mice. We established several pedigrees showing heritable sleep/wakefulness abnormalities. Among them, the Sleepy mutant pedigree shows 30% reduction in 24-h wake time. To map a chromosomal region responsible for the sleep phenotype of Sleepy mutant mice, we performed a linkage analysis in N2 mice, obtained by backcrossing the mutagenized founder C57BL/6J male to C57BL/6N female mice for two generations. The analysis revealed a single peak with a LOD score of more than 20. Whole exome sequencing of mutants and wild-type littermates from the Sleepy pedigree identified a nucleotide change specific to Sleepy mutant mice within the mapped chromosomal region. The single nucleotide substitution abrogates a splice donor site of the gene that we termed Sleepy. RT-PCR analysis of the brain and liver mRNA found a short variant of Sleepy mRNA specific to Sleepy mutant mice. Functional analyses of the Sleepy gene are now underway.

Disclosures: H. Funato: None. **C. Miyoshi:** None. **M. Sato:** None. **A. Ikkyu:** None. **N. Hotta:** None. **M. Kakizaki:** None. **S. Kanno:** None. **K. Harano:** None. **F. Asano:** None. **T. Fujiyama:** None. **T. Suzuki:** None. **S. Wakana:** None. **M. Yanagisawa:** None.

Nanosymposium

291. Sleep and Memory

Location: 206

Time: Monday, November 17, 2014, 8:00 AM - 10:45 AM

Presentation Number: 291.02

Topic: E.08. Biological Rhythms and Sleep

Title: Identification of a molecular mechanism for daytime-dependent hippocampal memory performance

Authors: *O. RAWASHDEH[1], J. FAHRENKRUG[2], J. H. STEHLE[1];
[1]Inst. of Cell. and Mol. Anatomy, Dr. Senckenbergische Anatomie III, Goethe Univ. Frankfurt,

Frankfurt am Main, Germany; [2]Dept. of Clin. Biochemistry, Bispebjerg Hosp., Univ. of Copenhagen, Copenhagen NV, Denmark

Abstract: It is well documented across different animal models and learning paradigms that memory processes (acquisition, consolidation and retrieval) are dependent on daytime, and modulated by a circadian clock. However, the mechanism(s) behind the temporal gating of learning and memory remain unknown. Our recent findings demonstrate that daytime-dependent dynamics in signaling and epigenetic modifications in mouse hippocampus are reliant and influenced by the clockwork component *PERIOD1* (*PER1*)[1,2]. *In vivo*, behavioral analyses revealed that day/night differences in the long-term improvement of spatial working memory performance are absent in mice deficient for *Per1*[2]. Concomitantly, we found that the endogenous rhythm in the phosphorylation of the transcription factor CREB (cyclic adenosine monophosphate responsive element binding protein), essential for long-term memory persistence, is PER1-dependent[2]. We therefore hypothesize that the core clockwork component PER1 is mechanistically central for the temporal modulation of hippocampus-dependent memory processes. Indeed, we reveal that PER1 is necessary for day/night differences in learning induced CREB activation, likewise to the here presented daytime-dependent pharmacological activation of this 'memory molecule' in hippocampal slices. Furthermore, we elucidate the molecular mechanism underlying the PER1-dependent regulation of CREB activation. Our data open a novel molecular facet in the role of clock genes as modulators that drive the daytime-dependent efficiency in spatial memory performance, an essential adaptive behavior for the survival of animals within their natural habitat. 1. Jilg et al., Hippocampus 2010, Mar;20(3):377-88. 2. Rawashdeh et al., Hippocampus 2014, Feb. 18, doi:10.1002/hipo.22262.

Disclosures: O. Rawashdeh: None. **J. Fahrenkrug:** None. **J.H. Stehle:** None.

Nanosymposium

291. Sleep and Memory

Location: 206

Time: Monday, November 17, 2014, 8:00 AM - 10:45 AM

Presentation Number: 291.03

Topic: E.08. Biological Rhythms and Sleep

Support: NSERC Grant N00097

Title: A complex processive stressor, conditioned fear, disrupts PERIOD1 expression in a manner similar to systemic stressors, in the rat central extended amygdala

Authors: *S. AL-SAFADI, A. AL-SAFADI, M. BRANCHAUD, S. RUTHERFORD, A. DAYANANDAN, B. ROBINSON, S. AMIR;
Concordia Univ., Montreal, QC, Canada

Abstract: Stressful events can disrupt circadian rhythmicity resulting in physiological and behavioral disturbances, but mechanisms underlying this disruption remain largely unknown. It is thought that these effects are brought about by stress-induced changes in circadian clock protein expression in brain regions that are vulnerable to stress and anxiety. We recently found that the category of stress, time-of-day and mode of stress exposure can modulate the expression of the core clock protein PERIOD1 (PER1), in the forebrain. Specifically, systemic stressors (interleukin-1β, 2-Deoxy-D-glucose) increased the expression of PER1 in the central extended amygdala (CeA) and other brain nuclei studied, while processive stressors (restraint, forced swimming), which induce an acute emotional response, suppressed protein levels exclusively in the CeA, suggesting that the suppression of PER1 expression in this region might be a distinct molecular signature of stressful stimuli that have emotional significance. Furthermore, we demonstrated a role for glucocorticoids and their receptors in the mediation of stress-induced changes in PER1 expression in the CeA. Here, we used contextual fear conditioning to investigate the effect of a complex processive stressor, conditioned fear, on PER1 expression in the CeA. Adult male Wistar rats were subjected daily to an aversive unconditioned stimulus, footshock stress (15 randomized shocks over 5 min, 1mA/s), at zeitgeber time (ZT) 2 for 4 days. On Day 5, the rats were re-exposed to the context previously paired with footshock. Blood and brains were collected 1 h post-stress onset at ZT3. We found that exposure to a context previously paired with footshock led to significant increases in PER1 levels in the CeA, but not in the suprachiasmatic nucleus, the master pacemaker. Changes in PER1 expression induced by the conditioned fear context resembled those induced by footshock itself, whereas PER1 expression in conditioned rats tested in a novel context not paired with footshock was not affected, demonstrating the robustness and specificity of the conditioned fear context. Contrary to our expectation, we found that the emotional state of fear elicits an effect on PER1 expression that is in fact characteristic of systemic not processive, stress, highlighting the complex nature of stress circuitry on clock gene expression. Moreover, these findings underscore the efficacy of the emotional state of fear in modifying clock protein expression in regions of the brain involved in stress, motivation and emotion, and suggest PER1 as a possible mediator linking fear and circadian mechanisms in the brain.

Disclosures: S. Al-Safadi: None. **A. Al-Safadi:** None. **M. Branchaud:** None. **S. Rutherford:** None. **A. Dayanandan:** None. **B. Robinson:** None. **S. Amir:** None.

Nanosymposium

291. Sleep and Memory

Location: 206

Time: Monday, November 17, 2014, 8:00 AM - 10:45 AM

Presentation Number: 291.04

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant P01-AG017628.02

Title: Chronic sleep fragmentation leads to pro-apoptotic signaling in locus coeruleus of aged mice

Authors: *A. STERN, N. NAIDOO;
The Univ. of Pennsylvania, Philadelphia, PA

Abstract: Sleep disturbances are a common concern among the elderly population, with older individuals frequently reporting nocturnal awakenings and daytime sleepiness resulting in a fragmented sleep/wake cycle. However, little is known regarding the impact of sleep fragmentation on neuronal health over aging. Using a mouse model, we showed previously that aging impairs the adaptive component of the unfolded protein response (UPR) – a quality control mechanism that is critical for maintaining protein homeostasis – specifically in response to sleep deprivation. We also demonstrated a particular vulnerability of wake-active locus coeruleus neurons, which in aged but not young mice showed evidence of pro-apoptotic signaling in response to acute sleep fragmentation. Here, we extend these experiments to determine the effects of chronic sleep fragmentation on protein homeostasis and the unfolded protein response in aged locus coeruleus, with a particular focus on pro-inflammatory and pro-apoptotic signaling pathways.

Disclosures: A. Stern: None. **N. Naidoo:** None.

Nanosymposium

291. Sleep and Memory

Location: 206

Time: Monday, November 17, 2014, 8:00 AM - 10:45 AM

Presentation Number: 291.05

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant 1 R01 MH60670-11

Department of Anesthesiology, University of Michigan Medical School

Title: Total sleep deprivation in rats for 8 hours using an automated air puff system does not increase activity of the hypothalamic-pituitary-adrenal axis

Authors: *B. A. GROSS[1], D. DAVIS[2], C. FITZPATRICK[3], K. PRABHU[2], L. URPA[2], G. R. POE[2];
[1]Univ. Michigan, Ann Arbor, MI; [2]Anesthesiol., [3]Neurosci. Grad. Program, Univ. of Michigan, Ann Arbor, MI

Abstract: Full sleep deprivation via gentle handling is time-consuming and can result in increased personnel costs. We have previously demonstrated that our automated air puff sleep deprivation system accurately detects sleep and delivers air puffs with sufficient pressure and temporal resolution to result in total sleep deprivation. The objective of this project was to show that the system is not any more stressful than gentle handling. Six male Sprague-Dawley rats were each implanted with cortical screw EEG and nuchal EMG electrodes connected to a 6-pin head cap. In addition, an indwelling intravenous catheter was implanted into the right external jugular vein with an access port implanted between the shoulder blades. This allowed for multiple blood samples to be taken over time so that each animal served as its own control. Blood samples were taken every 4 hours over the course of the rats' 12-hour light cycle on a baseline day, a day with 8 hours of total sleep deprivation via air puffs, and a day of 8 hours sleep deprivation via gentle handling. Offline manual stage scoring confirmed that normal sleep took place on the baseline day and that sleep deprivation occurred on the two manipulation days. Results from the immunoassay of isolated serum samples showed no significant differences in levels of ACTH, corticosterone, and melatonin (stress hormones indicative of HPA axis activity) at any circadian matched time point between baseline, sleep deprivation via air puffs, or gentle handling. These results, along with previously presented results on the accuracy and effectiveness of our automated air puff sleep deprivation system, suggest that it is a viable alternative to existing sleep deprivation techniques (i.e., gentle handling, multiple platforms over water, noise, and forced locomotion).

Disclosures: B.A. Gross: None. **D. Davis:** None. **K. Prabhu:** None. **L. Urpa:** None. **G.R. Poe:** None. **C. Fitzpatrick:** None.

Nanosymposium

291. Sleep and Memory

Location: 206

Time: Monday, November 17, 2014, 8:00 AM - 10:45 AM

Presentation Number: 291.06

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant HL 079555

Title: Wake neuron degeneration and lasting wake impairments in chronic sleep disruption

Authors: *S. C. VEASEY[1], Y. ZHU[2], L. YU[3], G. ZHAN[1], P. FENIK[1];
[1]Univ. Pennsylvania, PHILADELPHIA, PA; [2]Univ. of Pennsylvania, philadelphia, PA; [3]Norman Bethune Col. of Med., Changchun, China

Abstract: Chronic sleep disruption (CSD) commonly occurs in many sleep disorders; yet whether CSD has any lasting impact on brain health is largely unexplored. Wakefulness is modulated, in part, by collections of neurons throughout the brain that are activated in wakefulness and quiescent in sleep. We hypothesized that CSD-induces repeated excitation of wake-activated neurons (WAN) across sleep, which in turn imposes metabolic stress and ultimately leads to neuron loss. To examine this, we implanted adult mice with sleep-recording electrodes and exposed mice to either 14 wks of CSD (induced arousals 1/minute) or control rested conditions. Following exposures mice were allowed a two-week recovery of normal rested conditions. While there were no differences in 24hr wake, non-rapid-eye movement sleep (NREM) or rapid-eye-movement sleep (REMS) times, there were greater sleep/wake transitions, $p<0.01$, and in all behavioral states a slowing of EEG spectra was observed. Sleep latency was also reduced in CSD mice (9.7±1.1mins versus rested (12.7±1.1min, $p<0.05$) supporting a lasting increased sleep propensity. Using optical fractionator stereology we determined total locus coeruleus and orexinergic neuron counts in the two groups, finding reductions in both in CSD mice. Locus coeruleus neuron counts were Rested, 1862±119 and CSD, 1130±132, $p<0.001$, and orexinergic neurons were Rested, 2990±100 and CSD 2242±117, $p<0.001$. We next began exploring mechanisms by which CSD induces neuron loss and wake impairments. TNF-α is implicated in wake impairments in CSD but mechanisms have not been elucidated. It is assumed that microglia are the source of TNF-α. In light of significant injury to wake-activated neurons, we examined all wake-activated neuron groups for TNF-α immunoreactivity. To our surprise TNF-α was localized to all wake-activated neurons and not in glia in CSD, and only minimally present in brains of rested mice ($p<0.0001$). In CSD relative to rested mice, TNF-α (normalized to VDAC1 mitochondrial protein) was increased in the mitochondrial subcellular fraction (10.4±1 vs. 6.1±0.5, $p<0.01$). In summary, CSD results in a loss of orexinergic and locus coeruleus neurons, and we would anticipate that other wake-activated neurons are also affected. Importantly there are behavioral consequences, with residual wake impairments. With significant loss of locus coeruleus and orexinergic neurons, and prominent neuroinflammation, CSD is likely to be an important disease modifier for many neurodegenerative processes.

Disclosures: **S.C. Veasey:** None. **Y. Zhu:** None. **P. Fenik:** None. **G. Zhan:** None. **L. Yu:** None.

Nanosymposium

291. Sleep and Memory

Location: 206

Time: Monday, November 17, 2014, 8:00 AM - 10:45 AM

Presentation Number: 291.07

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant GM081259

NIH Grant MH099730

Title: Restoration of phosphorylated eukaryotic translation initiation factor 4E binding protein 2 (4EBP2) in the hippocampus rescues memory impairment due to sleep deprivation

Authors: *J. H. CHOI, E. J. DAVIS, R. HAVEKES, T. ABEL;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Sleep loss produces deficits in hippocampal synaptic plasticity and hippocampus-dependent memory storage. However, the molecular and cellular mechanisms that underlie these effects of sleep deprivation remain unclear. Previous work from our laboratory demonstrated that a prominent effect of even brief periods of sleep deprivation is attenuation of mammalian target of rapamycin (mTOR) signaling in the hippocampus. Specifically, five hours of total sleep deprivation reduces phosphorylated eukaryotic translation initiation factor 4E binding protein 2 (4EBP2) that subsequently leads to impaired protein synthesis. However it is yet to be determined whether restoring downstream mTOR signaling in the hippocampus is sufficient to prevent the cognitive deficits associated with sleep deprivation. To address this important question, we developed an adeno-associated virus (AAV) with a CamKII alpha promoter fragment to induce expression of 4EBP2 selectively in excitatory neurons of the hippocampus. Mice were bilaterally injected with 4EBP2 AAV and mice injected with enhanced green fluorescent protein (eGFP) AAV served as controls. Three weeks after hippocampal AAV infection, mice were trained in the hippocampus-dependent object place recognition task. Afterwards, mice were sleep deprived for five hours or left undisturbed in their home cage. We found that hippocampal overexpression of 4EBP2 resulted in increased phosphorylated 4EBP2, which was sufficient to prevent the memory deficits associated with sleep deprivation in the object place recognition task. These findings indicate that attenuated phosphorylated 4EBP2 levels in the hippocampus and subsequent impaired protein synthesis is the critical component underlying the memory deficits associated with sleep deprivation in hippocampus-dependent learning tasks. Furthermore, this study defines the molecular mechanism by which loss of sleep

impairs cognitive processes and highlights a vital role for translation and mTOR activation on long-term memory formation.

Disclosures: **J.H. Choi:** None. **E.J. Davis:** None. **R. Havekes:** None. **T. Abel:** None.

Nanosymposium

291. Sleep and Memory

Location: 206

Time: Monday, November 17, 2014, 8:00 AM - 10:45 AM

Presentation Number: 291.08

Topic: F.02. Animal Cognition and Behavior

Support: NS 19904

Title: Sleep promotes memory retention by blocking dopamine neuron based forgetting

Authors: ***J. A. BERRY**, I. CERVANTES-SANDOVAL, R. L. DAVIS;
The Scripps Res. Inst. Florida, Jupiter, FL

Abstract: Early psychological studies suggest that forgetting primarily occurs through subsequent mental activity that interfere with the stability of the previously formed memory and, furthermore, sleep could be beneficial to memory retention by blocking this activity (Wixted, 2004; Jenkins and Dallenbach, 1924). Recently in Drosophila, we showed that forgetting of olfactory memories is regulated by the subsequent ongoing activity of dopamine neurons (DANs) that innervate the Mushroom body (MBs) olfactory memory center (Berry et al., 2012). We hypothesized that this ongoing DAN activity could be regulated by the behavioral state of the animal. To test this, we designing a novel in vivo imaging assay allowing simultaneous measurement of the DAN activity and the physical activity of a fly as it ran on a ball supported by air. Intriguingly, we found that ongoing DAN activity impinging upon the MBs increases robustly during periods of activity and conversely diminishes when the animal rests. Furthermore, inducing sleep or quiescence in flies, either through administration of the sleep inducing drug Gaboxadol or through TrpA1 stimulation of the fan shaped body sleep circuit, significantly decreases DAN activity, while significantly enhancing memory retention. Therefore, we offer novel evidence that forgetting is regulated through behavioral state modulation of dopaminergic signaling and that sleep is beneficial to memory retention by blocking this dopamine neuron signal. Wixted, J.T. 2004. The psychology and neuroscience of forgetting. Annu. Rev. Psychol. 55, 235-269. Jenkins, J.B., Dallenbach, K.M. 1924. Oblivescence during sleep and waking. Am. J. Psychol. 35, 605-612. Berry, J.A., Cervantes-

Sandoval, I., Nicholas, E.P., Davis, R.L. 2012. Dopamine is required for learning and forgetting in Drosophila. Neuron 74, 530-542.

Disclosures: **J.A. Berry:** None. **I. Cervantes-Sandoval:** None. **R.L. Davis:** None.

Nanosymposium

291. Sleep and Memory

Location: 206

Time: Monday, November 17, 2014, 8:00 AM - 10:45 AM

Presentation Number: 291.09

Topic: F.02. Animal Cognition and Behavior

Support: NIH Predoctoral Neuroscience training grant 5T32MH019929-18

NIH Predoctoral Genetics training grant 5T32GM7122-37

NIH R01 MH067284

NINDS NRSA Predoctoral fellowship 1F31NS086764-01

Title: Functional connectivity between the dorsal paired medial neurons and the mushroom bodies in the *Drosophila* memory circuit

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Abstract: The mushroom bodies (MBs) in the Drosophila brain are important structures for learning and memory that integrate sensory experiences to regulate behavior much like the mammalian hippocampus, providing a powerful model for investigating memory consolidation. Behavioral studies have suggested that the MBs are modulated by a set of cells known as the dorsal paired medial neurons (DPMs), and these neurons have been shown to be necessary for memory consolidation. Although the cellular mechanisms underlying the DPMs' involvement in memory consolidation have not been characterized, it is currently thought that memory consolidation occurs via an excitatory recurrent feedback loop between the DPMs and subsets of MB neurons. To investigate this model, we utilized Drosophila genetic tools to activate either the DPMs or MBs by expressing ATP-gated purinergic (P2X2) receptors. Using live imaging on dissected brains, we bath-applied ATP and observed the response of the second set of cells via expression of genetically encoded molecular sensors. We first expressed P2X2 receptors in the

DPMs and confirmed that the ATP was effective at evoking calcium influx, voltage increase, and neurotransmitter release from these cells; however, DPM activation did not cause an increase in either calcium or cAMP in the MBs. Because these results are inconsistent with the current model of memory consolidation, we further characterized the DPMs by staining for various known neurotransmitters. We found evidence that the DPMs are GABAergic and serotonergic, but no evidence that these cells express excitatory neurotransmitters. These results indicate that a different mechanism is involved in the DPM modulation of the MBs than what has been previously suggested. One possibility is that the DPMs have an inhibitory effect on the MBs; however, current imaging techniques are inadequate for observing physiological levels of inhibition. We are currently developing an imaging technique for observing inhibition in this circuit, and preliminary results support a model of DPM inhibition of MB neurons. We also investigated the reverse functional connection by activating the MBs and observing the DPM response. Preliminary results show that MB activation evokes an increase in voltage and calcium in the DPMs. Although these combined results indicate that further research is needed to develop a more accurate model of memory consolidation, we propose a model in which learning-induced MB activation drives DPM activity. DPM activity may then have an inhibitory effect on specific subsets of MB neurons, which could serve to selectively dampen initial MB activity and stabilize memories during consolidation.

Disclosures: B.L. Christmann: None. **P.R. Haynes:** None. **L.C. Griffith:** None.

Nanosymposium

291. Sleep and Memory

Location: 206

Time: Monday, November 17, 2014, 8:00 AM - 10:45 AM

Presentation Number: 291.10

Topic: F.02. Animal Cognition and Behavior

Support: NSF Grant DGE-1256259

NIH Grant T32-GM007507

NIH Grant NS35575

NIH Grant HL/AR59649

NIH Grant NS063245

NIH Grant R21DA015753

Title: The clock gene period differentially regulates sleep and memory in *Drosophila*

Authors: *R. FROPF, J. C. P. YIN;
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Abstract: Circadian regulation is a conserved phenomenon across the animal kingdom, the disruption of which can have severe behavioral and physiological consequences. Likewise, core circadian clock proteins are well conserved from Drosophila to humans. While the molecular clock interactions that regulate circadian rhythms have been extensively described, additional roles for clock genes during complex behaviors are less understood. The clock gene period (per) has been studied in several Drosophila behavioral paradigms. Per null mutants and RNAi directed against per have been shown to disrupt memory while overexpression of wild-type per has been shown to rescue these memory impairments and enhance memory in wild-type flies. Though the memory effects of per manipulations are postulated to result from changes in sleep regulation, this theory has not been explicitly tested. Here, we show that per manipulations cause differential, time-of-day dependent changes in memory capacity. Per manipulations also result in altered sleep regulation, even while light/dark entrained. However, overall sleep cannot be linked to memory performance due to the differential memory capacity at different times of day. We are currently searching for distinct anatomical bases for per function in these different behaviors. These analyses of sleep and memory when per is manipulated highlight the importance of time-of-day- or arousal-dependent effects on molecular manipulations of memory.

Disclosures: R. Fropf: None. **J.C.P. Yin:** None.

Nanosymposium

291. Sleep and Memory

Location: 206

Time: Monday, November 17, 2014, 8:00 AM - 10:45 AM

Presentation Number: 291.11

Topic: E.08. Biological Rhythms and Sleep

Support: NIH R00 NS060996

NIH R01 NS070911

NIH R01 DA031367

NIH F32 NS082010

Della Martin Fund

Title: Hypocretin and norepinephrine interactions in zebrafish larvae

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Abstract: The hypothalamic neuropeptide hypocretin is a major regulator of wakefulness in vertebrates, including the diurnal teleost zebrafish. The neurotransmitter norepinephrine, supplied to the central nervous system by efferent projections of the locus ceruleus, has been shown to promote arousal in many vertebrate model systems. Here we employ a pharmacological approach to investigate the interactions between hypocretin and norepinephrine in larval zebrafish. We have previously shown that overexpression of hypocretin from a heat-shock system (HS-Hcrt) induces an insomnia-like state. Conversely, we find that the alpha-adrenergic antagonist prazosin increases sleep during both day and night. When HS-Hcrt larvae are subjected to heat shock, we find that the majority of the insomnia phenotype is inhibited by prazosin. These, as well as other, pharmacological studies suggest that norepinephrine is a major mediator of the hypocretin system in larval zebrafish, similar to what has been shown in other vertebrates. Thus zebrafish could provide a new diurnal vertebrate system for investigating the interactions between arousal promoting centers in the CNS.

Disclosures: G. Oikonomou: None. **C. Singh:** None. **D.A. Prober:** None.

Nanosymposium

292. Reward: Physiology and Connectivity

Location: 150B

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 292.01

Topic: A.10. Adolescent Development

Support: NIH:1R01 DA020269-01

Title: Resting state connectivity in adolescent marijuana users

Authors: *D. A. YURGELUN-TODD[1,2], M. P. LOPEZ-LARSON[1,2];
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Abstract: Background: Marijuana (MJ) is the most commonly used illicit drug by adolescents in the United States and several lines of investigation suggest that the integrity of the prefrontal circuitry may be particularly salient for understanding both the risk for onset of marijuana abuse and the transition into chronic use. Furthermore, brain changes during adolescence, particularly in the orbitofrontal cortex (OFC), have been suggested to increase risk for drug use and dependence by disrupting normal maturation and reward sensitivity. The aim of this study was to measure functional connectivity associated with frontal regions in MJ using adolescents and non-using healthy comparison subjects (HC). Methods: Forty three MJ users and 31 HC ranging in age from 14-20 years old completed clinical assessments and structural and functional imaging sequences on a 3T Siemens Trio scanner. MJ users reported at least 100 minimum lifetime smoking events in the previous year. Information regarding age of first MJ use, age of regular use, and frequency of MJ use was collected. BOLD echo planar images were obtained during an 8-minute resting state. FMRI images were analyzed using SPM8 (Wellcome Department of Imaging Neuroscience, University College, London, UK) and statistical threshold was set at $p < .005$, FDR corrected, and $k > 20$ voxels. Results**:** MJ users showed significantly greater functional connectivity than HC between the right and left OFC seeds and the cingulate and right middle frontal gyrus, right superior frontal gyrus and left precentral gyrus. Lifetime MJ exposure was associated with significant connectivity changes to regions associated with the limbic network and default mode network (DMN) whereas age of onset of MJ use was associated with greater right OFC functional connectivity to brain regions associated with motor movement. Conclusion: Adolescent MJ users demonstrated hyperconnectivity between the OFC and the anterior frontal-motor brain regions that support attention/executive function and motor planning functions. These connectivity changes may reflect neuromaturational abnormalities in OFC-mediated brain networks that are specific to MJ misuse. Lifetime exposure to marijuana was associated with additional OFC functional connectivity changes to posterior motor, DMN and limbic brain regions. These results suggest that in adolescents, both age of onset and exposure to cannabis impact brain circuits involved in cognition and mood.

Disclosures: D.A. Yurgelun-Todd: None. **M.P. Lopez-Larson:** None.

Nanosymposium

292. Reward: Physiology and Connectivity

Location: 150B

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 292.02

Topic: A.10. Adolescent Development

Support: NIAAA grant AA020033

Title: Intrinsic functional connectivity of dorsal versus ventral striatum in healthy adolescents: Age-related trends and behavioral correlates

Authors: *M. LUCIANA[1], P. F. COLLINS[1], J. CAMCHONG[2];
[1]Psychology, [2]Psychiatry, Univ. of Minnesota, Minneapolis, MN

Abstract: The striatum and its interconnections with frontal regions is implicated in motivation, flexibility, and decision-making. Models of human striatal organization propose a dorsal vs. ventral functional dissociation of circuitry that supports such behaviors. The dorsal striatum, via connections to prefrontal as well as posterior cortical regions, primarily mediates high level cognition. The ventral striatum mediates motivation and reward valuation through interconnections with orbitofrontal, insular, and anterior cingulate regions. These networks may show distinct developmental trends such that ventral circuitry peaks in activation in the adolescent period; this peak may underlie increases in risk-taking behavior. Here, we examine the development of the intrinsic functional connectivity (iFC) of the dorsal versus ventral striatum in a longitudinal study of healthy adolescents and young adults. Typically developing individuals (n=170; ages 11 to 25) were longitudinally studied at two year intervals across an 8 year period on a combined behavioral and neuroimaging protocol. Participants were free of psychopathology. Resting state scans were collected on a 3T Siemens Tim Trio scanner and processed using procedures outlined by the 1000 Functional Connectomes Project. Striatal iFC was measured by placing spherical seeds in the dorsal (caudate/putamen) and ventral striatum (ventral caudate/nucleus accumbens), respectively. Repeated neurobehavioral assessments included measures of working memory, inhibitory control, and flexibility as well as approach and avoidance motivation. Preliminary cross-sectional analyses indicate (a) differential patterns of dorsal versus ventral iFC that map onto extant models; (b) unique age-related associations with iFC in each broad region; and (c) evidence of age-related change that extends well into the second decade of life. Specifically, age appears to exert a stronger influence over ventral versus dorsal iFC with quadratic developmental trends evident in several regions of interest. Behavioral correlates also support a functional dissociation of striatal circuits. Longitudinal analyses are underway to confirm these findings. These distinct developmental trajectories of ventral versus dorsal striatal iFC in typically developing adolescents and young adults are informative regarding circuit-specific vulnerabilities that may underlie various forms of psychopathology that emerge during adolescence. Moreover, our findings suggest that striatal circuitry is developmentally plastic well into the second decade of life, supporting current theoretical models of adolescent brain development.

Disclosures: **M. Luciana:** None. **P.F. Collins:** None. **J. Camchong:** None.

Nanosymposium

292. Reward: Physiology and Connectivity

Location: 150B

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 292.03

Topic: A.10. Adolescent Development

Title: Development of the intrinsic functional connectivity of reward and salience circuitries

Authors: *M. ERNST[1], B. BENSON[1], P. KUNDRU[1], W.-M. LUH[2], P. BANDETTINI[3], D. S. PINE[3];
[1]NIMH/NIH, NIMH-NIH, BETHESDA, MD; [2]MRI facility, Cornell Univ., Ithaca, NY; [3]NIH, Bethesda, MD

Abstract: Characterizing the typical development of endogenous brain organization is a crucial complement to task-based activation studies to understand vulnerabilities to psychopathology in critical developmental time windows. We present data on resting state intrinsic functional connectivity (iFC) in 51 healthy subjects (24 adolescents, M=12.6 yo; 27 adults, M=29.3 yo), using a state-of-the-art acquisition multi-echo sequence. This method significantly reduces physiological and motion artifacts, which have been plaguing iFC studies, particularly in children. Based on documented developmental changes in reward and emotion processes, we first addressed the question of iFC of these related circuitries. Predictions were based on two main premises. First, sensitivity to reward stimuli is thought to be heightened in youths vs. adults, suggesting that the reward circuitry might be more active and thus characterized by higher iFC in younger ages. Second, the top-down modulation of somatovegetative and emotional processes increase with age, suggesting a strengthening of this circuitry (i.e., salience network) with increasing age. To test these hypotheses, we examined the modulation by age of whole-brain iFC of key nodes of the reward and salience networks, the ventral striatum and insula, respectively. Preliminary results are reported for the right lateralized seeds. The nucleus accumbens (MNI 9, 10, -11) showed significant iFC decrease with age to bilateral caudate, thalamus, and posterior cingulate. The insula (MNI 26, -4, 9) exhibited significant iFC decrease with age to putamen and superior temporal cortex, but also significant iFC increase with age to right DLPFC, bilateral DMPFC, and right inferior parietal. These findings are overall consistent with predictions, and they will be examined in association with behavioral characteristics.

Disclosures: M. Ernst: None. **B. Benson:** None. **P. Kundru:** None. **W. Luh:** None. **P. Bandettini:** None. **D.S. Pine:** None.

Nanosymposium

292. Reward: Physiology and Connectivity

Location: 150B

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 292.04

Topic: F.01. Human Cognition and Behavior

Support: NIDA Grant DA03369

Title: Opposite patterns of amygdala and ventral striatum reactivity predict stress-related risk of alcohol use disorders

Authors: *Y. S. NIKOLOVA, A. R. HARIRI;
Duke Univ., Durham, NC

Abstract: Background: Using data from the first 200 participants in the ongoing Duke Neurogenetics Study (DNS), we recently demonstrated that stress-related problem drinking is particularly pronounced in individuals with relatively high reward-related ventral striatum (VS) reactivity and relatively low threat-related amygdala reactivity (Nikolova & Hariri, 2012). This pattern may reflect an increased drive to pursue rewards coupled with a reduced ability to detect the potentially harmful consequences of drinking. In the current study, we extend these earlier findings by exploring a second putative profile of neural circuit function associated with stress-related problem drinking in a larger sample of DNS participants. We further probe potential affective mediators of these effects. Methods: Amygdala and VS reactivity were measured using well-characterized face-matching and number-guessing BOLD fMRI paradigms, respectively, in an expanded sample of 759 DNS participants (426 women, mean age 19.65 ± 1.24). Recent stress, problem drinking and depressive symptoms were assessed via self-report. Results: We extend prior findings by demonstrating that stress is associated with problem drinking not only in individuals with relatively high VS and low amygdala reactivity, but also in those with relatively low VS and high amygdala reactivity (p's<0.02). Stress does not, however, correlate with problem drinking in individuals whose VS and amygdala reactivity are either both high or both low (p's >0.5). We further show that current depressive symptoms mediate the relationship between stress and alcohol use for those with low VS and high amygdala reactivity, but not for those with the opposite combination of neural traits. Conclusions: Our findings support the existence of two distinct neural profiles associated with stress-related problem drinking: one associated with an increased drive to pursue rewards and reduced threat sensitivity, possibly reflecting drinking for stimulation and positive emotion enhancement; and one associated with decreased reward drive and increased sensitivity to threat, possibly reflecting drinking to reduce negative emotion. Our findings also suggest the existence of two distinct protective patterns of

neural functioning: one where threat and reward responsiveness are both high, and another where they are both low.

Disclosures: **Y.S. Nikolova:** None. **A.R. Hariri:** None.

Nanosymposium

292. Reward: Physiology and Connectivity

Location: 150B

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 292.05

Topic: F.01. Human Cognition and Behavior

Title: COMT and serotonin transporter alleles as predictors of hypnotic susceptibility

Authors: *K. WANNIGMAN[1], R. BLACKWELL[2], E. GAHTAN[3];
[2]Biol., [3]Psychology, [1]Humboldt State Univ., Arcata, CA

Abstract: Several gene variants have broad psychological effects. The serotonin transporter (SERT) gene S and LG alleles, associated with slower transcriptional activation of the gene, are linked to stress vulnerability and poorer responses to Cognitive Behavioral Therapy. The Catechol-O-methyltransferase (COMT) gene Met allele, associated with slower dopamine metabolism, is linked to stress vulnerability and poorer attention. Since these cognitive and psychological traits also relate to hypnosis, we asked whether SERT and COMT genotype modifies hypnotic susceptibility. COMT genotype has previously been shown to modify hypnotic susceptibility, though results have been inconsistent with regard to the effects of specific alleles. No studies have directly examined the associations between the SERT genotype and hypnotic susceptibility. We predicted that S/LG and Met carriers would show lower hypnotic susceptibility compared to individuals with alternative gene variants (LA or Val, respectively), and that the effects of the two genes would be additive. Two hundred and fifty three participants (155 female, ages 18-69) donated buccal cells for SERT and COMT genotyping and completed the Harvard Group Scale for Hypnotic Susceptibility Form A (HGSHS-A) - a 45 min hypnotic procedure designed to measure susceptibility. Participants also completed the Tellegan Absorption Scale (TAS), which has been used previously as an index of hypnotic susceptibility but with mixed evidence of validity. Distribution of COMT alleles in the sample were in line with estimates of allele frequency in the general populations, with 28% of the sample (n=70) Val/Val, 47% (n=119) Val/Met, and 25% (n=64) Met/Met. Of 12 hypnotic suggestions in the HGSHS-A, the mean response number was 7.2±2.5, and the range was 0-12. Although Val carriers had higher mean hypnotic susceptibility scores (Val/Val=7.19, Val/Met=7.26,

Met/Met=6.92) as expected, the association between COMT genotype and HGSHS-A scores was not significant. Therefore, we were unable to replicate findings linking COMT and hypnotic susceptibility despite using a larger sample than previous studies. There was no association between the TAS and the HGSHS-A ($r = .067$, $p = .144$), suggesting the TAS is not a valid indicator of hypnotic susceptibility. SERT genotyping for this study is in progress. Hypnotherapy is an empirically supported treatment for a wide variety of mental, physical, and emotional dysfunctions, but its therapeutic value could be enhanced by reliable predictors of individual responses. Whether genotyping can provide such predictors remains to be determined.

Disclosures: K. Wannigman: None. **R. Blackwell:** None. **E. Gahtan:** None.

Nanosymposium

292. Reward: Physiology and Connectivity

Location: 150B

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 292.06

Topic: F.01. Human Cognition and Behavior

Title: The structural scaffolding of functionally defined networks predicts individual differences in cognitive function

Authors: *N. REGGENTE, J. RISSMAN;
Dept. of Psychology, UCLA, Los Angeles, CA

Abstract: Efforts to map the structural and functional connectivity of the human brain promise to offer insights into the neural underpinnings of cognition. A growing body of work has shown how various neurological and psychiatric disorders are characterized by abnormal manifestations of inter-regional connectivity within specific networks. Furthermore, recent studies have demonstrated that functional connectivity patterns within cortical networks can be used to reliably decode information pertaining to subjects' cognitive task-sets. However, an account of the relationship between cognitive task performance and the structural integrity of the networks purportedly necessary for such tasks has yet to be vigorously pursued. Thereby, in the current investigation we sought to assess the degree to which the anatomical strength of connections within task-specific networks relates to cognitive performance differences across a population of healthy subjects. Using diffusion-weighted MRI images made available by the Human Connectome Project, we implemented probabilistic tractography algorithms to obtain pairwise metrics of anatomical connectedness within previously defined functional networks (Power et al., 2011). We then used these structural network connectivity values as predictors in a support

vector regression analysis in order to predict individual differences in cognitive functions. Using a leave-one-subject-out cross-validation framework, the model's predictions were able to account for a significant percentage of the variance in performance on tasks hypothesized to be functionally associated with each network. Specifically, we found that the structural network connectivity profile of the cingulo-opercular network significantly predicted subjects' fluid intelligence and executive function scores; connectivity within the fronto-parietal control network predicted spatial orientation ability; and connectivity within the memory retrieval network predicted performance on an episodic memory task. Network-behavior specificity was illustrated by a lack of converging predictive power (i.e., no individual network predicted performance on all tasks). These results suggest that the underlying integrity of structural connections that make up functionally defined networks can at least partially account for an individual's ability to execute tasks that allegedly depend on such networks.

Disclosures: **N. Reggente:** None. **J. Rissman:** None.

Nanosymposium

292. Reward: Physiology and Connectivity

Location: 150B

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 292.07

Topic: F.01. Human Cognition and Behavior

Support: Fred B. Snite Foundation

Title: Meta-analysis of sex difference in human hippocampal volume

Authors: ***L. S. ELIOT**, A. TAN, W. MA, A. VIRA, R. ADAMCZYK;
Dept. Neurosci., Rosalind Franklin Univ. Med. & Sci., North Chicago, IL

Abstract: Hippocampal atrophy is found in many CNS disorders, such as depression and Alzheimer's disease, that show higher prevalence in women. Sex differences in memory and spatial skills further suggest that males and females may differ in hippocampal structure and function (Andreano & Cahill, 2009). To test whether the hippocampus is reliably sexually dimorphic, we conducted meta-analyses of reported hippocampal volumes in matched samples of healthy males and females of all ages. Using four search strategies, we found 59 MRI studies that reported mean $\pm$ SD of raw or uncorrected hippocampal volume in 65 matched samples of males and females, and 33 studies that reported hippocampal volumes in 36 gender-matched samples after correcting for individual differences in overall brain or intracranial volume. All volumetric

data were converted to Hedges g values and pooled effect sizes were calculated using a random-effects model for left, right, and bilateral raw hippocampal volumes and for left, right, and bilateral hippocampal volumes corrected for overall sex difference in total brain or intracranial volume. Each dataset was further tested for heterogeneity and meta-regressed against study year and average participant age. We found that uncorrected hippocampal volume is about 7% larger in males, with a pooled effect size of 0.545 for left hippocampus (50 independent samples), 0.526 for right hippocampus (49 samples), and 0.557 for total hippocampus (29 samples). Meta-regression revealed no effect of age on the sex difference in left, right, or bilateral hippocampal volume. In the subset of studies that reported it, total brain volume (TBV) was considerably larger in males (g=1.162, 21 samples) and similarly for total intracranial volume (ICV; g= 1.272, 22 samples). Accordingly, studies reporting hippocampal volumes corrected for individual differences in TBV or ICV revealed no significant sex differences in left and right hippocampal volumes (pooled Hedges' g values ranging from +0.011 to -0.206, p values ranging from 0.070 to 0.918, sample sizes ranging from 8 to 29 matched male/female groups). In a smaller collection of studies that reported corrected bilateral hippocampal volumes, females' volumes were significantly larger than males' (g=-0.361, p=0.004; 11 matched samples); however, the magnitude and significance of this effect depended on whether ICV or TBV was used as the correction factor. In summary, males of all ages exhibit larger hippocampal volume than females, but adjusting for individual differences in TBV or ICV largely eliminates this difference. The claim that females have a disproportionately large hippocampus compared to males is not supported.

Disclosures: L.S. Eliot: None. **A. Tan:** None. **W. Ma:** None. **A. Vira:** None. **R. Adamczyk:** None.

Nanosymposium

292. Reward: Physiology and Connectivity

Location: 150B

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 292.08

Topic: F.01. Human Cognition and Behavior

Title: Psychophysiological investigations of individual differences using eeg and fmri

Authors: *J. CIORCIARI[1], J. GOUNTAS[2];
[1]Brain & Psychological Sci. Res. Ctr., Swinburne Univ. of Technol., Melbourne, Australia;
[2]Murdoch Univ., Perth, Australia

Abstract: EEG and fMRI were used in two separate studies which investigated the psychophysiological correlates associated with personality and thinking styles using a personality orientations model based on the Jungian four personality functions; the Gountas Personality Orientation (GPO). The GPO measures the strength of each thinking style; Emotion (E), Material (M), Intuitive/Imaginative (I) and Logical (L). The EEG & fMRI studies examined functional distribution of networks and examined whether specific neural networks exist for each orientation. In the first EEG study, 43 participants were tested, while 40 were tested in the fMRI study. Functional activity were correlated with a series of language based tasks and introspection tasks. In support of the EEG study, the fMRI study demonstrated that each thinking style has its own functional neural network during introspection. These data suggest a left hemisphere relationship for the M and L types and a right neural network relationship for E and I types. The implications associated with the findings suggest that specific networks exit for personality related styles of thinking and introspective decision making.

Disclosures: J. Ciorciari: None. **J. Gountas:** None.

Nanosymposium

292. Reward: Physiology and Connectivity

Location: 150B

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 292.09

Topic: F.01. Human Cognition and Behavior

Support: Intramural Research Program, NIH grant 1ZIAHD000641 from the NICHD with supplemental funding from NIMHD, the Bench to Bedside Program and the Office of Behavioral and Social Sciences Research (OBSSR) of the NIH (to J. Yanovski & M. Tanofsky-Kraff)

USUHS grant R072IC (to M. Tanofsky-Kraff)

NIDDK grant 1R01DK080906 (to M. Tanofsky-Kraff)

Intramural Research Program, NICHD grant Z1AHD00641 (to J. Yanovski)

Intramural Research Program, NIMH (to D. Pine)

Title: Differences in inhibitory control in adolescents with and without loss of control (LOC) eating: A potential role of the dorsolateral prefrontal cortex (dlPFC) in motor inhibition and peer interaction

Authors: *D. M. BONGIORNO[1], A. VANNUCCI[2,3], E. E. NELSON[1], J. JARCHO[1], L. B. SHOMAKER[2,4], L. M. HANNALLAH[2,3], K. R. THEIM[3], S. E. FIELD[2], S. M. BRADY[2], C. K. PICKWORTH[2], M. V. GRYGORENKO[2], T. CONDARCO[2], A. P. DEMIDOWICH[2], D. S. PINE[1], M. TANOFSKY-KRAFF[2,3], J. A. YANOVSKI[2];
[1]Section on Develop. and Affective Neurosci., Natl. Inst. of Mental Hlth., Bethesda, MD; [2]Section on Growth and Obesity, Program in Developmental Endocrinol. and Genet., Natl. Inst. of Child Hlth. and Human Develop., Bethesda, MD; [3]Dept. of Med. and Clin. Psychology, Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD; [4]Colorado State Univ., Fort Collins, CO

Abstract: Pediatric loss of control (LOC) eating promotes excess weight gain, metabolic dysfunction, and worsening mood symptoms and is a precursor of exacerbated disordered eating. Some models propose that emotion dysregulation in social contexts – which may involve inhibitory control deficits – is an important risk factor for LOC eating. In Study 1, adolescents (N=98; 15.8±1.5y; 41% White, 31% Black, 28% Other) spanning broad weight strata (mean BMI-z: 1.0±0.9) completed the Stop Signal paradigm. Slower stop signal reaction times (SSRTs, ms) on the Stop Signal task are indicative of poorer inhibitory control. Presence (LOC+, n=28) or absence (LOC–, n=70) of LOC eating was determined by interview. There was no significant main effect of LOC on inhibitory control (SSRT; p=.38), but there was a significant LOC x BMI-z interaction (p=.02): slower SSRT was associated with higher BMI-z in LOC+, but with lower BMI-z in LOC–. In Study 2, we examined the interaction between inhibitory control and functional brain activity during a validated social stress paradigm in a subset of overweight and obese girls from Study 1 (LOC+, n=9; LOC–, n=9; mean-matched on age and BMI-z). Prior to undergoing neuroimaging, girls sorted photos of 60 female peers into two groups: those they selected or rejected for a future virtual chat. During an fMRI scan, social stress was evoked by reminding participants of the peers they selected or rejected and asking them to guess if each peer selected them for the chat. Prior studies have found that predicting such peer evaluations is a provocative psychosocial experience for adolescents. A 3-way ANOVA with factors for group status (LOC+ or LOC–), selection status (peer selected or rejected by participant), and SSRT score revealed a 3-way interaction for bilateral dlPFC activation. Decomposition analyses revealed that in the LOC– group, slower SSRT was associated with greater dlPFC activity when anticipating feedback from selected versus rejected peers. In the LOC+ group, no association with peer selection was found. This pattern of results suggests that LOC– youth, even those who behaviorally demonstrate poorer inhibitory control, may appropriately engage cognitive control resources while anticipating potentially stressful peer evaluations. This anticipatory neural response appears to be either absent or dysfunctional in LOC+ youth. Taken together, findings from both studies suggest deficits in inhibitory control may underlie weight problems in youth with LOC eating. Prospective data are needed to determine if inhibitory control deficits and dysfunctional dlPFC engagement in the context of social stress promote obesity in youth with LOC eating.

Disclosures: **D.M. Bongiorno:** None. **A. Vannucci:** None. **E.E. Nelson:** None. **J. Jarcho:** None. **L.B. Shomaker:** None. **L.M. Hannallah:** None. **K.R. Theim:** None. **S.E. Field:** None. **S.M. Brady:** None. **C.K. Pickworth:** None. **M.V. Grygorenko:** None. **T. Condarco:** None. **A.P. Demidowich:** None. **D.S. Pine:** None. **M. Tanofsky-Kraff:** None. **J.A. Yanovski:** None.

Nanosymposium

292. Reward: Physiology and Connectivity

Location: 150B

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 292.10

Topic: A.10. Adolescent Development

Title: Risky decision-making in adolescent girls: The role of gonadal hormones at puberty

Authors: *Z. A. OP DE MACKS[1], S. BUNGE[1], L. KRIEGSFELD[1], A. KAYSER[2], R. DAHL[1]; [1]UC Berkeley, Berkeley, CA; [2]UCSF, San Fransisco, CA

Abstract: This study examines the role of pubertal development, reward magnitude, and social comparison in adolescent risky decision-making and associated brain processes. In a revised version of the Jackpot task (Op de Macks et al., 2011), 67 healthy, female adolescents aged 11-13 years chose to "play" the game or "pass" based on information about risk (low/high), reward magnitude (small/large), and type of feedback (social ranking/money). Decisions to "play" were influenced by risk and reward magnitude, and were associated with increased activation in reward-related brain regions (e.g., midbrain and ventral striatum). Salivary gonadal hormone assessments (n = 57) revealed that girls with higher testosterone levels made more decisions to "play" in the high-risk, small-reward condition within social feedback blocks ($p = .007$); this relationship was marginal within monetary feedback blocks ($p = .079$). A whole-brain regression analysis revealed increased ventral striatum activation (cluster-corrected at $p < .05$, FWE) for girls with higher testosterone levels when playing in the social vs. monetary feedback condition. These findings suggest that the relationship between hormonal changes at puberty and brain processes involved in risky decision-making may be amplified in a social context, which supports previous evidence for increased reward processing in the context of risky decision-making when adolescents are in the presence of peers (Chein et al., 2010). Future analyses will focus on understanding the role of estradiol in risky decision-making and associated brain processes.

Disclosures: **Z.A. Op De Macks:** None. **S. Bunge:** None. **L. Kriegsfeld:** None. **A. Kayser:** None. **R. Dahl:** None.

Nanosymposium

293. Extended Amygdala Circuits and Behavior

Location: 150A

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 293.01

Topic: F.02. Animal Cognition and Behavior

Support: AA020140

DA19112

AA019455

Title: Multiple modes of noradrenergic modulation of glutamatergic transmission onto corticotropin releasing factor neurons in the bed nucleus of the stria terminalis

Authors: ***Y. SILBERMAN**, D. G. WINDER;
Vanderbilt Univ. Sch. of Med., Nashville, TN

Abstract: Stress-induced reinstatement of drug-seeking behaviors is thought to be due to norepinephrine (NE) modulation of corticotropin releasing factor (CRF) signaling in the bed nucleus of the stria terminalis (BNST). We previously found that NE recruits CRF signaling to enhance excitatory drive in the BNST via β-adrenergic receptor (AR) activation while α-ARs inhibit overall excitatory drive in this brain region. The mechanism by which NE may modulate excitatory drive directly onto BNST CRF neurons is not yet known. These studies utilized whole-cell patch-clamp electrophysiology methods in a novel genetic reporter strategy to explore AR control of BNST CRF neurons. Acute application of NE to BNST slices significantly inhibited the amplitude of evoked excitatory postsynaptic currents (EPSCs) recorded from CRF neurons with variable changes to paired pulse ratios (PPR), suggesting multiple AR subtypes may be present in glutamatergic synapses of BNST CRF neurons. Bath application of the α1-AR agonist methoxamine significantly reduced EPSC amplitude in BNST CRF neurons without altering PPR suggesting a postsynaptic role of α1-ARs. Similarly, the α2-AR agonist guanfacine significantly reduced EPSC amplitude in BNST CRF neurons but in this case increased PPR suggesting a presynaptic role of α2-ARs at these synapses. Conversely, the β-AR agonist isoproterenol increased EPSC amplitude and did not alter PPR. Together, these findings suggest

that in naïve mice, norepinephrine inhibits overall glutamatergic transmission at BNST CRF neurons via α1 and α2-AR activation which masks β-AR mediated excitation of these synapses. Experiments are currently in progress to determine the effect of chronic drug exposure at these synapses. Overall, these studies suggest testable hypotheses regarding mechanisms involved in the development of negative reinforcement-based drug related behaviors.

Disclosures: Y. Silberman: None. **D.G. Winder:** None.

Nanosymposium

293. Extended Amygdala Circuits and Behavior

Location: 150A

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 293.02

Topic: F.02. Animal Cognition and Behavior

Support: LIA CNRS-NYU LearnEmoTime

PUF grant Emotion and Timing

Title: Ontogeny of molecular changes in the amygdala induced by odor-shock learning

Authors: *L. DIAZ-MATAIX[1], E. SANTINI[1], E. C. SARRO[2,3,5,6], R. E. PERRY[2,4,6,7], L. TALLOT[8], J. E. LEDOUX[1,2], E. KLANN[1], V. DOYERE[8], R. M. SULLIVAN[2,3,6,7]; [1]Ctr. for Neural Sci., New York Univ., NEW YORK, NY; [2]Emotional Brain Inst., Nathan Kline Inst., Orangeburg, NY; [3]Child and Adolescent Psychiatry,, [4]Child and Adolescent Psychiatry, New York Univ. Sch. of Med., New York, NY; [5]Neurosci. and Physiol., NYU Sackler Inst. for Grad. Biomed, New York, NY; [6]NYU Child Study Ctr., NYU Langone Med. Ctr., New York, NY; [7]Neurosci. and Physiol., NYU Sackler Inst. for Grad. Biomed. Sci., New York, NY; [8]Ctr. of Neurosciences Paris-Sud (CNPS), Univ. Paris XI/CNRS, Orsay, France

Abstract: In rat pups, odor-shock conditioning induces odor preference from birth to post-natal day 9 (PN9), being critical to develop mother-pup attachment and therefore ensure survival. After this age, odor-shock conditioning results in odor aversion. However it is not known whether animals at these ages are able to consolidate odor-shock memories. In adults, mTOR and ERK signaling pathways regulate protein synthesis and the synaptic plastic changes occurring in the amygdala in order to consolidate threat memories. The objective of this work is to study if odor aversion learning following odor-shock conditioning is accompanied by age-dependent alterations in mTOR and/or ERK signaling pathways. Five different groups of animals (PN7-9;

PN11-13; PN18-20; PN24-26; adults) were odor conditioned by pairing peppermint odor with a mild hind limb shock. For the behavioral experiments memory was tested 24 hours after conditioning. For the biochemistry experiments animals were sacrificed 15 minutes after the last conditioning trial and the brains were rapidly frozen for later dissection of the amygdala. As expected, animals aged PN11-13 were the youngest to display avoidance of conditioned odor when memory was tested 24 hours after learning. In line with the behavioral results, conditioning-induced phosphorylation in the mTOR and ERK pathways in the amygdala increased with the age of the animals. The results show that odor-shock memory consolidation occurs in parallel with changes in the mTOR and ERK pathways that are critical for plasticity in the amygdala.

Disclosures: L. Diaz-Mataix: None. **E. Santini:** None. **E.C. Sarro:** None. **R.E. Perry:** None. **R.M. Sullivan:** None. **L. Tallot:** None. **V. Doyere:** None. **E. Klann:** None. **J.E. LeDoux:** None.

Nanosymposium

293. Extended Amygdala Circuits and Behavior

Location: 150A

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 293.03

Topic: F.02. Animal Cognition and Behavior

Support: NIA

Title: Neural circuitry underlying extinction of trace fear conditioning

Authors: *M. SEHGAL[1], T. S. BULA[1], N. B. FETTINGER[1], J. R. MOYER, Jr.[1,2]; [1]Psychology, Univ. of Wisconsin-Milwaukee, Milwaukee, WI; [2]Biol. Sci., Univ. of Wisconsin-Milwaukee, MIlwaukee, WI

Abstract: With an increasing proportion of the world population constituting the elderly, aging-related cognitive decline has an immense socio-economic impact. Such cognitive decline includes cognitive flexibility deficits whereby elderly individuals are impaired on learning that a previously learned rule is no longer valid. One example of cognitive flexibility is extinction learning where animals learn that a previously learned conditioned stimulus (CS) is no longer predictive of the unconditioned stimulus (US). Recently, our lab has demonstrated that aging rodents are impaired on extinction of trace fear conditioning (Kaczorowski, 2012). Extensive evidence suggests that extinction of delay fear conditioning relies on opposing activity within

medial prefrontal cortex sub-regions, infralimbic (IL) and prelimbic cortex (PL). While PL activity promotes fear expression, IL activity promotes successful extinction. However, relatively little is known about the neural circuitry that underlies extinction of trace fear conditioning and how this circuit changes during the course of normal aging. In fact few, if any, studies have investigated how normal aging alters learning-related changes in neuronal activity within a distributed memory circuit. The current experiments aim to understand the neural circuitry that mediates trace fear extinction and evaluate how this circuit changes during normal aging. Briefly, rats were divided into 4 groups; naïve, unpaired, trace fear conditioned (TRACE) or extinction (EXT) group. On day 1, TRACE and EXT group received 10 paired CS-US presentations (white noise CS, 1.3mA footshock, 30s trace interval). The unpaired group received unpaired presentations of 10 CS and US each. On days 2 and 3, rats in the EXT as well as unpaired group received 10 CS presentations alone. On day 4, fear memory was tested in the TRACE, EXT, and unpaired group using 2 CS-alone presentations. After the CS-test, brains were removed and processed for immunohistochemistry to quantify the expression of a variety of signaling molecules, including immediate early gene, Zif-268. Preliminary results from adult rats demonstrate that trace fear conditioning significantly increases the number of Zif-268 labeled neurons within PL. This effect was reversed by extinction. Surprisingly, similar results were obtained within IL - Zif-268 labeling was increased following trace fear conditioning but not extinction. Ongoing experiments (data not yet analyzed) are investigating changes in neuronal activation following trace fear conditioning and extinction in aging rats as well as neuronal activity changes in other brain regions that underlie extinction learning.

Disclosures: **M. Sehgal:** None. **T.S. Bula:** None. **N.B. Fettinger:** None. **J.R. Moyer:** None.

Nanosymposium

293. Extended Amygdala Circuits and Behavior

Location: 150A

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 293.04

Topic: F.02. Animal Cognition and Behavior

Support: NIH R0115841

Title: Real-time measurement of norepinephrine dynamics in the ventral bed nucleus of the stria terminalis during drug withdrawal and aversive stimuli

Authors: ***R. WIGHTMAN**[1], M. E. FOX[2], E. S. BUCHER[2];
[2]Chem., [1]Univ. North Carolina, Chapel Hill, NC

Abstract: Drug-dependence is thought to occur during the termination of drug-use when an individual experiences the negative affect of withdrawal. A number of psychological disorders are comorbid with substance abuse, and signaling within the bed nucleus of the stria terminalis (BNST) has been suggested to be a site of this convergence. Previously, we used fast-scan cyclic voltammetry (FSCV) to demonstrate robust noradrenergic plasticity in animal models of addiction. When Sprague Dawley rats were made morphine dependent, they exhibited reduced control over norepinephrine release in the BNST, accompanied by increased anxiety-like behavior. Drug-dependent Sprague-Dawley rats were neurochemically, and behaviorally indistinguishable from Lewis rats, a model of enhanced drug-seeking and anxiety. Here, we use FSCV to make measurements during acute morphine withdrawal in awake animals. These rapid, real-time measurements allow us to elucidate potential mechanisms by which this plasticity occurs. We found norepinephrine is released during naloxone-precipitated withdrawal in discrete events, a contrast to our previous work measuring diffuse norepinephrine release during infusion of an aversive tastant. Release tracked the time course of somatic withdrawal behaviors, and electrically evoked norepinephrine following precipitated withdrawal was attenuated as compared with control rats. Similar norepinephrine "depletion" was found in animals exposed to an aversive electrical stimulus. Our data demonstrate changes in norepinephrine signaling during drug withdrawal and aversive stimuli that may be important for future drug dependence, escalation, and reinstatement.

Disclosures: R. Wightman: None. **M.E. Fox:** None. **E.S. Bucher:** None.

Nanosymposium

293. Extended Amygdala Circuits and Behavior

Location: 150A

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 293.05

Topic: F.02. Animal Cognition and Behavior

Support: F31-AA022280-01 (to NAC)

NARSAD (to TLK)

R01 5-30778 (to TLK)

INIA U01 532401 (to TLK)

DoD 5-55624 (to TLK)

Title: Dynorphin controls the gain of an amygdalar anxiety circuit

Authors: *N. A. CROWLEY[1], T. KASH[2];
[2]Pharmacol., [1]UNC, Chapel Hill, NC

Abstract: Dynorphin controls the gain of an amygdalar anxiety circuit The Bed Nucleus of the Stria Terminalis (BNST) plays a key role in regulation of stress and addiction related behavior. Kappa opioid receptors (KORs) and their endogenous ligand dynorphin are located throughout the brain, including the BNST, and modulate aversive or anxiogenic related behaviors. However, the relationship between the dynorphin-KOR system and BNST function and behavior is unknown. We used a multi-facetted approach to probe the ability of the BNST dynorphin-KOR system to modulate circuit function and anxiety-related behavior. We found, using whole-cell electrophysiology recordings, that KOR activation inhibited glutamate inputs from the basolateral amygdala (BLA), but not the prefrontal cortex (PFC), demonstrating pathway specificity of KOR modulation. Further, using converging approaches, we demonstrated a presynaptic locus of function of this effect. We then found that this form of KOR modulation was p38 MAP kinase and calcium-dependent. In a series of parallel experiments, we examined the nature of these changes and their relationship to KOR-dependent changes in anxiety-like behavior. We transfected the BLA with an AAV encoding a CamKii-driven ChR2, and implanted an optical fiber above the dorsolateral BNST. We found that light-activation of the BLA-BNST pathway produces an anxiolytic effect in multiple assays, and that this phenotype is blocked by administration of a KOR agonist. We next investigated the potential source of dynorphin to the BNST using a combination of anatomy and electrophysiology. Our results indicate that KOR dependent modulation of glutamatergic transmission in the BNST is due primarily to dynorphin release from local neurons in the BNST. Taken together, this data supports the conceptual model that dynorphin released from BNST neurons activates KOR on BLA inputs to the BNST act to enhance anxiety by suppressing glutamate transmission.

Disclosures: **N.A. Crowley:** None. **T. Kash:** None.

Nanosymposium

293. Extended Amygdala Circuits and Behavior

Location: 150A

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 293.06

Topic: F.02. Animal Cognition and Behavior

Support: NFS Grant RUI1050256

UW-Whitewater Undergraduate Research Grant

Title: The extended amygdala also mediates appetitive states

Authors: *M. A. WARACZYNSKI, A. SCHULTZ;
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Abstract: Over the past several years our lab has employed the phenomenon of brain stimulation reward (BSR) to show that the extended amygdala (EA) mediates appetitive state in addition to its well documented role in aversive states such as fear, stress, anxiety, and drug withdrawal. Past data have shown that temporary inactivation and other pharmacological manipulations of the EA, particularly the sublenticular EA (SLEA), reduce the stimulation pulse frequency required to maintain half-maximal rates of responding for BSR. Guided by information gained from the study of GABAergic medium spiny neurons (MSN's) in the striatum - neurons similar to those found throughout the EA - we have shown that dopamine D2 receptor stimulation combines with AMPA glutamate receptor blockade in the SLEA to reduce BSR's reward efficacy, while D1 blockade does not play as strong a role. New data to be presented here show that challenging SLEA-resident MSN activity by blocking L-type calcium ($Ca_V1.3$) channels via intracerebral injection of the phenylalkylamine verapamil or the benzothiazapine diltiazem reduces BSR's reward effectiveness, sometimes for more than 24 hours. Given that D2 receptor stimulation indirectly blocks L-type calcium channels through phospholipase C -inositol triphosphate-calcineurin signaling, our previous success at impairing BSR through D2 stimulation may have worked through this mechanism. Thus, the appetitive state engendered by BSR appears to rely at least in part on activation of MSN's in the SLEA. Aversive and appetitive state converge on the process of reinforcement of adaptive behavior. Negative reinforcement promotes adaptive behaviors that alleviate, avoid, or eliminate aversive states produced by threats to survival while positive reinforcement promotes interaction with stimuli that enhance survival. Our data invite other investigators to consider that (a) the EA may be involved in adaptive behaviors mediated by both negative and positive reinforcement; (b) elements of the EA besides the bed nucleus of the stria terminalis are important to this function and (c) knowledge gleaned from the study of MSN ensembles in the striatum may successfully guide investigation of the EA's role in both aversive and appetitive states.

Disclosures: M.A. Waraczynski: None. **A. Schultz:** None.

Nanosymposium

293. Extended Amygdala Circuits and Behavior

Location: 150A

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 293.07

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant AA018400

NIH Grant AA021013

Title: High traumatic stress reactivity is associated with escalated alcohol drinking and altered stress peptides in prefrontal-amygdala circuitry

Authors: *N. W. GILPIN[1], E. A. ROLTSCH[1], B. B. BAYNES[1], A. M. WHITAKER[1], B. A. BAIAMONTE[1], Y.-L. LU[2], H. N. RICHARDSON[2];
[1]Louisiana State Univ. Hlth. Sci. Ctr., New Orleans, LA; [2]Univ. of Massachusetts, Amherst, MA

Abstract: Some (but not all) humans exposed to traumatic stress develop a psychiatric disorder that is defined by high avoidance of trauma-related stimuli, hyperarousal, and negative affect, and that is highly co-morbid with Alcohol Use Disorder (AUD). Our lab uses a predator odor stress model in which animals that exhibit high traumatic stress reactivity (indexed by avoidance behavior) also exhibit persistent increases in alcohol drinking and altered prefrontal cortex (PFC)-amygdala neuronal activation profiles in response to stress-related stimuli. Here, we utilized this predator odor stress model to examine stress effects on corticotropin-releasing factor (CRF)-positive cell counts in ventromedial prefrontal cortex (vmPFC) and central amygdala (CeA). We also utilized systemic and brain site-specific pharmacology to examine the role of CRF-1 receptors (CRF1Rs) in mediating stress-induced escalation of alcohol drinking and hyperalgesia. In all experiments, odor-exposed rats were indexed for avoidance of a predator odor-paired context and divided into "Avoiders" (i.e., high stress reactivity) and "Non-Avoiders" (i.e., low stress reactivity), as previously published by our lab. Avoider rats have more CRF-positive cells in vmPFC 9 days following predator odor exposure, as measured by immunohistochemistry. Radioimmunoassay (RIA) revealed an increase in total CRF peptide in CeA of Avoider rats 21 days post-stress. Systemic antagonism of CRF1Rs by R121919 reverses stress-induced increases in alcohol drinking, arousal, and thermal nociception. Avoider rats exhibit post-stress hyperalgesia, considered to be an indicator of negative affect, that is reversed by systemic antagonism of CRF1Rs, and this drug effect is contingent on an intact and functional CeA. Furthermore, intra-CeA CRF infusion mimics stress-induced thermal hyperalgesia, and this effect is reversed by both CRF1R and GABA-A receptor antagonists. Our results suggest that dysregulation of brain and behavior by traumatic stress can be predicted based on stress reactivity profiles. Rats that exhibit high stress reactivity also exhibit escalated alcohol drinking, hyperalgesia, lasting changes in CRF cell counts in prefrontal cortex-amygdala circuitry, and altered sensitivity to CRF1R antagonist. These results suggest that CRF1Rs may be a promising pharmacotherapeutic target for treatment of co-morbid traumatic stress disorders and Alcohol Use Disorder.

Disclosures: **N.W. Gilpin:** None. **E.A. Roltsch:** None. **B.B. Baynes:** None. **A.M. Whitaker:** None. **B.A. Baiamonte:** None. **Y. Lu:** None. **H.N. Richardson:** None.

Nanosymposium

293. Extended Amygdala Circuits and Behavior

Location: 150A

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 293.08

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01-DA006214

NIH Grant K99-DA035251

Title: Extended amygdala modulates midbrain dopamine populations during cocaine seeking

Authors: ***S. V. MAHLER**[1], G. ASTON-JONES[2];
[1]Neurosciences, Med. Univ. of South Carolina, CHARLESTON, SC; [2]Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: Although extended amygdala (EA) is best known for its roles in anxiety and stress, EA also regulates appetitive motivation, including conditioned seeking for cocaine and other rewards. Here, we will review our recent data showing roles for BNST, central nucleus of the amygdala, and striatopallidal subregions in cocaine seeking behaviors. In particular, we emphasize that EA modulation of midbrain dopamine neurons is important for conditioned cocaine seeking. We conclude that projections from EA and related structures to the ventral tegmental area are crucial for Pavlovian cues to elicit drug seeking. We also note that careful examination of heterogeneity within EA subregions is necessary to understand the precise roles for EA subregions, and their projections to midbrain, in addiction. Supported by PHS Grants R01-DA006214, K99-DA035251

Disclosures: **S.V. Mahler:** None. **G. Aston-Jones:** None.

Nanosymposium

293. Extended Amygdala Circuits and Behavior

Location: 150A

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 293.09

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant AA5-33266

Title: Examining the influence of central amygdale neurotensin neurons on ethanol behaviors

Authors: *Z. A. MCELLIGOTT, P. KANTAK, S. FACCIDOMO, G. PATEL, C. HODGE, G. STUBER;
Univ. of North Carolina, Chapel Hill, Chapel Hill, NC

Abstract: The central nucleus of the amygdala (CeA), a heterogeneous nucleus that contains several different genetically and electrophysiologically defined cell types, has been identified as a critical node for the reinforcing properties of ethanol self-administration. Recent studies have demonstrated that ethanol disinhibits a certain subset of neurons within the CeA, however, additional studies are needed to examine the circuitry that could mediate these effects. One of these genetically defined populations are the neurons expressing the 13 AA neuropeptide neurotensin (NTS). Several studies have suggested that the NTS system contributes to drug seeking and anxiety like behaviors, however, much of this work has focused on the mesolimbic dopamine system. To begin to explore the function of NTS neurons in the CeA we used a genetically selective lesion strategy . Following the lesion we did not observe alterations in food/water consumption between lesioned and control animals. Following 6 weeks, we then tested the CeA-NTS lesioned animals and controls across anxiety measures. We did not observe any differences between the groups on the elevated plus maze or in the light-dark box but we did see a significant increase in time spent in the center of an open field. Furthermore we find that the CeA-NTS neurons regulate multiple ethanol behaviors and comprise an intriguing circuit for relaying ethanol related information.

Disclosures: Z.A. McElligott: None. **P. Kantak:** None. **S. Faccidomo:** None. **G. Patel:** None. **C. Hodge:** None. **G. Stuber:** None.

Nanosymposium

293. Extended Amygdala Circuits and Behavior

Location: 150A

Time: Monday, November 17, 2014, 8:00 AM - 10:30 AM

Presentation Number: 293.10

Topic: F.02. Animal Cognition and Behavior

Support: K01-MH083052

F31-MH102008

Title: Anatomical connectivity of the bed nucleus of the stria terminalis in humans

Authors: S. AVERY[1], J. CLAUSS[1], *J. U. BLACKFORD[2];
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Abstract: Background: The bed nucleus of the stria terminalis (BNST) is a small, relatively understudied region in the medial forebrain which mediates anxiety responses and addictive behavior. Much of what we know about anatomical connectivity of the BNST comes from tracer studies in rodents, and speculation about BNST connectivity in humans is derived from these studies. Therefore the primary goal of this study was to characterize BNST anatomical connectivity in humans. Additionally, in order to characterize individual differences in connectivity potentially related to psychopathology risk, we tested for associations between anxiety and addictions genes and white matter microstructure within identified anatomical pathways. Methods: We conducted an exploratory diffusion tensor imaging (DTI) analysis in 72 individuals (mean age = 31 years; 44% female). The BNST was seeded for probabilistic tractography to 55 cortical and subcortical brain regions per hemisphere, and anatomical connectivity was measured as the proportion of BNST tractography streamlines connecting with each brain region. Bootstrapping methods were used to identify regions with significant anatomical connectivity (> 95th percentile). In a subset of 32 individuals, serotonin pathway candidate single nucleotide polymorphisms and 5-HTTLPR were genotyped using the Sequenom platform and Taqman assay, respectively. Dose-dependent association between genotype and diffusion anisotropy (FA), a measure of white matter microstructure, were assessed using regression ($p<.01$). Results: The BNST showed significant anatomical connectivity with multiple limbic and frontal cortex brain regions, including the amygdala, hippocampus, accumbens, and orbital and medial frontal cortices. Higher FA values in the amygdalofugal pathway and uncinate fasciculus were associated with increased COMT, 5-HTR1B, and 5-HTTLPR minor allele frequency, while lower FA values in the stria terminalis were associated with increased COMT and 5-HT2A minor allele frequency. Conclusions: To our knowledge, this study is the first to characterize BNST anatomical connectivity in the human brain. Importantly, our results are similar to findings in rodents indicating that DTI is a method that can inform human BNST anatomical connectivity. We additionally show that individual differences in white matter microstructure in BNST pathways are associated with allelic variation in anxiety and addictions-related genes. Given the role of the BNST in anxiety and addiction, understanding BNST anatomical connectivity in humans is an important first step in identifying novel connectivity-based mediators of psychopathology risk.

Disclosures: **S. Avery:** None. **J.U. Blackford:** None. **J. Clauss:** None.

Nanosymposium

382. Disease Modeling Using Pluripotent Stem Cells I

Location: 156

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 382.01

Topic: A.04. Stem Cells

Support: CIRM Grant TR2-01832

CIRM Grant RB4-06277

Title: Modeling neurological diseases using patient ipscs

Authors: *Y. SHI, J. CHAO, W. LI, E. TIAN, P. YE, G. SUN;
Dept. of Neurosciences, Beckman Res. Inst. of City of Hope, Duarte, CA

Abstract: Recent developments in stem cell biology and cellular reprogramming have catalyzed unprecedented advances in disease modeling and drug discovery. The ability to derive distinct human cell types is enabling innovative approaches to create *in vitro* disease models. We have generated induced pluripotent stem cells (iPSCs) from patients of neurological diseases and differentiated these cells into functional neurons and astrocytes. These patient neural cells will be used to dissect the pathological mechanisms of devastating neurological diseases.

Disclosures: Y. Shi: None. **J. Chao:** None. **W. Li:** None. **E. Tian:** None. **P. Ye:** None. **G. Sun:** None.

Nanosymposium

382. Disease Modeling Using Pluripotent Stem Cells I

Location: 156

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 382.02

Topic: A.04. Stem Cells

Support: NIH grant PO1GM081621-01A1

1R01MH082068-01A2

CIRM (RB3-02129)

Ministry of Science and Technology of China

Natural Science Foundation of China

Title: Circuitry-dependent and independent phenotypes of Rett syndrome “disease-in-dish” models

Authors: *X. CHEN[1,2], X. HAN[3], B. BLANCHI[1], W. GE[1], X. ZHANG[2], Z. PANG[4], L. CHENG[5], T. SÜDHOF[6], Y. SUN[1], Y. YU[3];
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Abstract: The path of using human induced pluripotent stem cells (hiPSCs) to build novel neurological “disease-in-dish” models has not been lack of obstacles. One bottleneck is how to identify stable phenotypes that are disease relevant. Here we report that MeCP2-deficient neurons from Rett syndrome iPSCs as well as modified hESCs exhibit consistent impairment in maturation as indicated by immature action potentials and reduced spine densities. However, when spontaneous postsynaptic currents and ratios between excitation-inhibition were measured, we observed enormous variations. Using transcriptome analyses, we found while pan-neuronal features are rather stable in RTT “disease-in-dish” models, neuronal subtype-specific properties, hence the content of functional neural circuits, are highly variable. Intentional alterations of circuitry identities using sonic hedgehog to change neuronal subtypes, shifted the neural transmission phenotype, suggesting a circuitry-specific nature. Our findings revealed that neurotransmission phenotypes could be circuitry-dependent, which is probably a common feature for many neurological disorders and “disease-in-dish” models.

Disclosures: **X. Chen:** None. **X. Han:** None. **B. Blanchi:** None. **W. Ge:** None. **X. Zhang:** None. **Z. Pang:** None. **L. Cheng:** None. **T. Südhof:** None. **Y. Sun:** None. **Y. Yu:** None.

Nanosymposium

382. Disease Modeling Using Pluripotent Stem Cells I

Location: 156

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 382.03

Topic: A.04. Stem Cells

Support: NIH Grant R01NS048271

NIH Grant R01HD069184

NIH Grant R33MH087874

NIH Grant R37NS047344

NIH Grant F31MH102978

MSCRF

NARSAD

Title: Deficits in core synaptic signatures in a human iPSC model of major mental disorders with a 4bp-frame-shift mutation in DISC1 gene

Authors: *Z. WEN[1,2], H. NGUYEN[2,3], Z. GUO[5], M. A. LALLI[6], J. SHIN[1,3], X. WANG[2], Y. SU[1,2], N.-S. KIM[1,2], K.-J. YOON[1,2], C. ZHANG[1,2], R. MAGOLIS[3,4], G. CHEN[5], K. S. KOSIK[6], H. SONG[1,2], G.-L. MING[1,2];
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Abstract: Severe psychiatric illnesses, such as schizophrenia and major depression, are chronic and complicated neurological diseases with a prominent genetic basis. However, the causes of these mental disorders are still poorly understood due to the lack of a representative model that accurately recapitulates the nature and distribution of the human pathology. Human induced-pluripotent stem cells (iPSCs), which carry the genetic information from patients, pave the way to study human development and to discover the molecular and cellular basis of human diseases in a more tractable experimental system. Here we generated iPSCs from four members of a family in which a frame-shift mutation of Disrupted-in-schizophrenia 1 (DISC1) co-segregated with psychiatric disorders. We further produced different isogenic iPSC lines with TALEN genome editing technique. We show that mutant DISC1 leads to deficits in presynaptic vesicle release in human forebrain neurons. Mechanistically, mutant DISC1 causes transcriptional dysregulation of many genes related to synapses and psychiatric disorders and depletes wild-type DISC1. Furthermore, mechanism-guided pharmacological inhibition of phosphodiesterases rescues synaptic defects in mutant neurons. Our studies directly support the synapse hypothesis for the etiopathology of psychiatric disorders and uncover a novel mechanism through which the disease-relevant mutation affects synaptic functions via transcriptional dysregulation.

Disclosures: **Z. Wen:** None. **H. Nguyen:** None. **Z. Guo:** None. **M.A. Lalli:** None. **J. Shin:** None. **X. Wang:** None. **Y. Su:** None. **N. Kim:** None. **K. Yoon:** None. **C. Zhang:** None. **R. Magolis:** None. **G. Chen:** None. **K.S. Kosik:** None. **H. Song:** None. **G. Ming:** None.

Nanosymposium

382. Disease Modeling Using Pluripotent Stem Cells I

Location: 156

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 382.04

Topic: A.04. Stem Cells

Title: Generation of human spastin-deficient pluripotent stem cells and neurons to model hereditary spastic paraplegia *in vitro*

Authors: *K. DOBRINDT[1], M. PEITZ[1], L. SCHOELS[2], O. BRUESTLE[1];
[1]Inst. of Reconstructive Neurobio., Bonn, Germany; [2]Dept. for Neurodegenerative Diseases, Hertie Inst. for Clin. Brain Res., Tübingen, Germany

Abstract: Hereditary spastic paraplegia (HSP) is a rare, heterogeneous group of genetic disorders with progressive spasticity in the lower limbs caused primarily by axonal degeneration of corticospinal motor neurons. The most frequent type of autosomal dominant paraplegia, spastic paraplegia 4 (SPG4), represents about 40% of all HSP cases and is caused by mutations in the SPAST gene, which codes for the microtubule severing enzyme spastin. Here we report the generation of a SPG4 in vitro model based on patient-specific induced pluripotent stem cell (iPSC)-derived cortical cultures. To this end, fibroblasts of family members carrying identical heterozygous SPAST nonsense mutations were reprogrammed to pluripotency and subsequently differentiated into cortical cultures comprising >80% glutamatergic pyramidal neurons expressing layer V and VI markers CTIP2 and TBR1, respectively. Neuronal cultures were found to express more spastin compared to iPSCs, and spastin levels in both SPG4 iPSCs and neurons were reduced by approximately 50% compared to controls. We focused on the identification of early neuronal HSP-related phenotypes and observed that one-week-old HSP neurons subjected to passaging exhibit a significant decrease in neurite length already as early as 24 hours post plating. Within only five days of neuronal maturation, axonal swellings, a hallmark of HSP, could be detected at a frequency of 1.7/mm in SPG4 neurons (vs. 0.1/mm in control neurons). Axonal swellings ranged between 1 and 7 µm in diameter and stained positive for the axonal marker tau1, acetylated tubulin and mitochondria as well as partially for neurofilament. Overall, we expect the described rapid phenotypic assays to accelerate the study of

pathomechanisms underlying HSP as well as the identification of therapeutic compounds for the treatment of this disease.

Disclosures: **K. Dobrindt:** None. **M. Peitz:** None. **O. Bruestle:** None. **L. Schoels:** None.

Nanosymposium

382. Disease Modeling Using Pluripotent Stem Cells I

Location: 156

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 382.05

Topic: A.04. Stem Cells

Support: CIRM

Tau Consortium

NIH

Title: Genetic correction of tauopathy phenotypes in neurons derived from human induced pluripotent stem cells

Authors: *H. FONG[1,2], C. WANG[1,2], J. KNOFERLE[1,2], D. WALKER[1,2], M. BALESTRA[1,2], L. M. TONG[1,2], L. LEUNG[1,2], K. L. RING[1,2], Y. HUANG[1,2];
[1]J. David Gladstone Inst., San Francisco, CA; [2]Univ. of California, San Francisco, San Francisco, CA

Abstract: Tauopathies represent a group of neurodegenerative disorders characterized by the accumulation of pathological TAU protein in brains. We report a human neuronal model of tauopathy derived from induced pluripotent stem cells (iPSCs) carrying a TAU-A152T mutation. Using zinc-finger nuclease-mediated gene editing, we generated two isogenic iPSC lines: one with the mutation corrected, and another with the homozygous mutation engineered. The A152T mutation increased TAU fragmentation and phosphorylation, leading to neurodegeneration and especially axonal degeneration. In addition, a decrease in the number of dopaminergic neurons was observed in neurons carrying the mutation. These cellular phenotypes were consistent with those observed in a patient with TAU-A152T. Upon mutation correction, normal neuronal and axonal morphologies were restored, accompanied by decreases in TAU fragmentation and phosphorylation, while the severity of tauopathy was intensified in neurons with the homozygous mutation. These isogenic TAU-iPSC lines represent a critical advancement towards the accurate

modeling and mechanistic study of tauopathies with human neurons, and will be invaluable for drug-screening efforts and future cell-based therapies.

Disclosures: **H. Fong:** None. **C. Wang:** None. **J. Knoferle:** None. **D. Walker:** None. **M. Balestra:** None. **L.M. Tong:** None. **L. Leung:** None. **K.L. Ring:** None. **Y. Huang:** None.

Nanosymposium

382. Disease Modeling Using Pluripotent Stem Cells I

Location: 156

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 382.06

Topic: A.04. Stem Cells

Support: MSCRF

NARSAD

NIH Grant R01NS048271

NIH Grant R01HD069184

NIH Grant R33MH087874

NIH Grant R37NS047344

IMHRO

Title: Modeling subcortical band heterotopia using patient-derived cerebral organoids

Authors: ***C. ZHANG**[1], H. NGUYEN[2], K.-J. YOON[1], Z. WEN[1], J. SHIN[2], J. SHIM[1], J. THAKOR[1], X. QIAN[1], K. CHRISTIAN[1], G. KRAUSS[3], H. SONG[1], G.-L. MING[1];
[1]Inst. For Cell Engin., Baltimore, MD; [2]Grad. Program In Cell. and Mol. Med., Baltimore, MD; [3]Dept. of Neurol., Baltimore, MD

Abstract: The complexity of neurodevelopmental disorders has made it difficult to study in model organisms. The recent development of a human pluripotent stem cell-derived 3-D organoid culture system called cerebral organoids, provides an in vitro system from which to study cortical brain disorders resulting from malformations of human cortical development. Here we use iPSC-derived in vitro cerebral organoids to model a rare congenital brain disorder called subcortical band heterotopia (SBH), a heterotopic cortex underlying the normotopic cortex, in a

three-member family containing a male patient suffering from SBH with a posterior-anterior gradient. Blood sequencing confirmed that the patient does not have a mutation in the doublecortin (DCX) and LIS1 genes. Using sendai virus, we generated iPSCs from the fibroblasts of a male SBH patient and his apparently healthy mother and father. Small molecule inhibitors of BMP and TGF beta signaling were used to direct organoid development towards forebrain. Immunohistochemistry show that male patient-derived organoids at least 42 days old exhibit a phenotype of two distinct neuronal-like layers of TBR1/CTIP2/MAP2+ cells: a normotopic layer in the cortical plate and a heterotopic layer lying in SVZ/IZ region. Healthy controls have one layer in the cortical plate. Our study strongly suggests: 1.) A migrational defect in the cortical structures of male patient organoids reminiscent of clinical SBH 2.) the presence of a genetic mutation found in somatic cells causing SBH in the male patient. SBH cerebral organoids can be employed as a model system for identifying the causative mutation through genomic sequencing and as a drug screening platform.

Disclosures: **C. Zhang:** None. **H. Nguyen:** None. **K. Yoon:** None. **Z. Wen:** None. **J. Shin:** None. **J. Shim:** None. **J. Thakor:** None. **X. Qian:** None. **K. Christian:** None. **G. Krauss:** None. **H. Song:** None. **G. Ming:** None.

Nanosymposium

382. Disease Modeling Using Pluripotent Stem Cells I

Location: 156

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 382.07

Topic: A.04. Stem Cells

Title: Regulation of inhibitory synaptic transmission by hPEM-2 in human induced neuronal cells

Authors: ***S. CHANDA**[1], D. HAAG[2], C. E. ANG[2], N. YANG[2], J. KIM[3], Y. S. KIM[3], T. C. SÜDHOF[4], M. WERNIG[2];
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Abstract: Postsynaptic GABAA receptors (GABAARs) constitute the principal component of GABA-mediated fast inhibitory synaptic transmission in the central nervous system. One of the key regulatory proteins that have been proposed to control the submembrane localization of GABAARs is Collybistin, a postsynaptic guanine nucleotide exchange factor, but the underlying mechanisms remained poorly understood. The human homolog of Collybistin, hPEM-2, has been found to be mutated in several cases of chronic noncommunicable disorders like epilepsy and

hyperekplexia, but little is known about its role due to technical difficulties of studying human neurons. Here we investigate the functional importance of hPEM-2 and its cellular phenotype associated with pathogenic mutations in induced neuronal (iN) cells derived from human embryonic stem (hES) cells. Our data indicate that manipulation of endogenous hPEM-2 expression levels in hES-iN cells affects postsynaptic Gephyrin clustering and GABAAR localization. Furthermore, hEPM-2 specifically affected GABAAR-mediated but not AMPAR-mediated synaptic transmission, suggesting functional specificity of hPEM-2 at human inhibitory synapse. Future experiments outline molecular replacement of endogenous hPEM-2 with splice-variant, domain-deleted and point-mutated versions to respectively isolate isoform-specific and domain-specific physiological phenotypes as well as disease-specific pathological traits. Importantly, this system will allow implementing molecular manipulations in human inhibitory postsynaptic complex in a controlled fashion to understand mechanisms involved in inhibitory synaptogenesis and maintenance, which may enable future design of targeted pharmacological interventions in a systematic manner.

Disclosures: S. Chanda: None. **D. Haag:** None. **C.E. Ang:** None. **N. Yang:** None. **J. Kim:** None. **Y.S. Kim:** None. **T.C. Südhof:** None. **M. Wernig:** None.

Nanosymposium

382. Disease Modeling Using Pluripotent Stem Cells I

Location: 156

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 382.08

Topic: A.04. Stem Cells

Support: NIH R21NS081484

NICHD P30HD03352

Title: Cell-autonomous phenotypes in rett syndrome ipsc-derived astrocytes

Authors: *Q. DONG, Q. CHANG;
Waisman Ctr., Univ. of Wisconsin-Madison, Madison, WI

Abstract: Mutations in the X-linked human methyl-CpG binding protein 2 (MECP2) gene have been identified as the genetic cause of Rett syndrome (RTT), a devastating neurodevelopmental disorder that predominantly affects females. Studies using genetically engineered mice lacking all or part of the Mecp2 gene have contributed to the majority of our current understanding of the

molecular mechanism of RTT. Recently, our lab and others have generated patient-specific induced pluripotent stem cell (iPSC) lines from RTT patients carrying various disease-causing mutations, and demonstrated that RTT iPSCs and their derivatives can serve as complimentary experimental systems for studying the disease mechanism. Our RTT iPSC system is particularly unique in that the wild type control and mutant lines are derived from the same patient. This isogenic feature helps to minimize phenotypic variation across iPSC lines and improve the sensitivity in detecting genotype-dependent phenotypes. As one of the major cell types in the mammalian nervous system, astrocytes play an important role in neurodevelopment and in diseases. Previous work from the Mandel lab has revealed a non-cell autonomous influence of MeCP2-deficient glia on neuronal dendritic morphology. Recently, we have differentiated human RTT iPSCs into glial fibrillary acidic protein expressing (GFAP+) astrocytes and observed similar non-cell autonomous effect on neuronal morphology. Moreover, we have discovered cell autonomous deficits in mutant RTT astrocytes, and begun to understand how MECP2 mutations may cause these phenotypes and how the cell autonomous deficit may be linked to the non-cell autonomous influence on neurons. Better understanding of the glial contribution to RTT pathology will not only provide insight into disease mechanisms, but also lay the groundwork for future drug screens using RTT iPSC-derived astrocytes.

Disclosures: Q. Dong: None. **Q. Chang:** None.

Nanosymposium

382. Disease Modeling Using Pluripotent Stem Cells I

Location: 156

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 382.09

Topic: A.04. Stem Cells

Support: NIH Grant NIMH MH099587.

Title: Astrocytes from ALS patient iPSCs result in neuron degeneration *in vivo*

Authors: *H. CHEN, K. QIAN, L. BLACKBOURN IV, A. ERRIGO, A. ERRIGO, Z. DU, S.-C. ZHANG;
Waisman Ctr., Madison, WI

Abstract: Amyotrophic lateral sclerosis (ALS) is a late-onset neurodegenerative disease characterized by a progressive loss of motoneurons. Astrocytes isolated from ALS transgenic mice or ALS patients, including those of sporadic ALS, have been shown to be toxic to motor

neurons in culture although the ways by which ALS astrocytes result in neuronal death is not clear. It is not known either if astrocytes without prior contact with the disease environment (e.g., from ALS patient iPSCs) cause neuron degeneration in vivo. We have established a chimeric mouse model in which human astrocytes replace mouse counterparts in the cervical spinal cord. In this model, we found that human astrocytes derived from sporadic ALS patients as well as healthy individuals migrated in the SCID mouse spinal cord, replaced endogenous astrocytes, and contacted neurons to a similar extent. However, ALS astrocytes exhibited a higher level of immunoreactivity to GFAP and reduced level of GLT1 as compared to non-ALS human astrocytes. Neurons in the vicinity of ALS but not non-ALS astrocytes, including motor neurons, displayed a decrease in number and size and an increase in immunoreactivity to phospho-neurofilament. Behavioral analysis revealed declined grip strength and forelimb movement deficits in the ALS astrocyte group by 9 months post-transplantation. Thus, astrocytes derived from sporadic ALS iPSCs, even without prior activation, can cause neuronal degeneration with functional outcomes. This suggests that astrocytes are a potential target for therapeutic intervention.

Disclosures: **H. Chen:** None. **K. Qian:** None. **L. Blackbourn IV:** None. **A. Errigo:** None. **A. Errigo:** None. **Z. Du:** None. **S. Zhang:** None.

Nanosymposium

382. Disease Modeling Using Pluripotent Stem Cells I

Location: 156

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 382.10

Topic: A.04. Stem Cells

Support: NIH NINDS R01 NS059546

RC1 NS068370

Regenerative Medicine grants TR2-01832

Regenerative Medicine grants RB4-06277

Title: Brain organoid and disease model

Authors: ***G. SUN**, Y. SHI;
City of Hope Beckman Res. Inst., DUARTE, CA

Abstract: The human brain, the structure basis of the intelligence and mind, is an extremely complex system and consists of extremely diverse types of neurons. This complexity has made studying the brain and decoding how it works a formidable task in neuroscience, especially for neurodegenerative diseases. Induced pluripotent cell (iPSC) technology offers tremendous hope for regenerative medicine, for its potential to provided unlimited sources of cells for disease modeling and drug discovery. Recently development of organoid techniques, by which the mini-organ can be derived from either IPSCs or embryonic stem cells, adds further excitement to this fascinating field. A novel protocol of producing miniature human brain was published recently. Our goal is to model human brain development and neurodegenerative diseases using this technology. In particular, we aim to identify novel disease phenotypes for Parkinson's disease at organ level, which cannot be detected at cellular level.

Disclosures: G. Sun: None. **Y. Shi:** None.

Nanosymposium

382. Disease Modeling Using Pluripotent Stem Cells I

Location: 156

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 382.11

Topic: A.04. Stem Cells

Support: JST, CREST

Research Center Network for Realization of Regenerative Medicine of the Japan Science and Technology Agency (JST)

Research on Applying Health Technology, the Ministry of Health, Labour and Welfare of Japan

Grant-in-Aid for Scientific Research on Innovative Area Foundation of Synapse and Neurocircuit Pathology (22110007) from the Ministry of Education, Culture, Sports, Science and Technology of Japan

Japan Research Foundation for Clinical Pharmacology

The Mochida Memorial Foundation for Medical and Pharmaceutical Research

Intramural Research Grant (24-9) for Neurological and Psychiatry Disorders of NCNP

Title: The use of iPS cells toward the treatment of neurodegenerative diseases

Authors: *H. INOUE[1,2];
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Abstract: The iPSC technology was established based on numerous findings by past and current scientists. Although the detailed mechanisms underlying the reprogramming process during iPSC generation are still being elucidated, the final products, which had previously been inaccessible, show promise for multiple purposes related to understanding disease mechanisms and treatment (Takahashi and Yamanaka, Development, 2013; Inoue et al., EMBO J. 2014). In neurodegenerative diseases, selective neurons are vulnerable and degenerate. The greatest risk for these diseases is aging, and every person faces this unavoidable risk. For the coming aging society, prevention and control of these diseases will be research and social imperatives. Various research efforts up to now have been contributing to advancing their outcome, including neuropathological findings, discoveries of causative genes with functional analysis, and the generation of model animals. However, solving the diseases still remains, especially sporadic cases, the majority of the diseases. For understanding the diseases correctly, analysis of the neural cells of the patients has been required. Now, iPS cell technology is able to provide us with patient neural cells. Using these new materials, we are modeling the diseases, analyzing the disease markers for patient stratification, or cell transplantation research.

Disclosures: H. Inoue: None.

Nanosymposium

382. Disease Modeling Using Pluripotent Stem Cells I

Location: 156

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 382.12

Topic: A.04. Stem Cells

Support: Project for the Realization of Regenerative Medicine and Support for Core Institutes for iPS Cell Research from the Ministry of Education, Culture

Support for the Core Institutes for iPS Cell Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT; to H.O.)

Grant-in-Aid for the Global COE Program from MEXT to Keio University

Grant-in-Aid for Young Scientists (B) from MEXT to Y.N

Keio University Grant-in-Aid for the Encouragement of Young Medical Scientists to from the Kanrinmaru-Project at Keio University to Y.O

Grant-in-Aid for Young Scientists (A) and a Grant-in-Aid for Scientific Research on Innovative Areas (Foundation of Synapse Neurocircuit Pathology) from MEXT to Y.O

JST-CIRM Collaborative Research Program funding awarded to Y.O

Title: Dysmyelination and enhanced ER stress response in Pelizaeus-Merzbacher disease patients iPSCs-derived oligodendrocytes with PLP1 gene missense mutations

Authors: *Y. NUMASAWA[1,2], Y. OKADA[3], S. SHIBATA[4], S. KAWABATA[5], N. KISHI[4], W. AKAMATSU[2], M. SHOUJI[6], A. NAKANISHI[6], H. OSAKA[7], K. INOUE[8], S. YAMANAKA[9], K. KOSAKI[10], M. NAKAMURA[5], T. TAKAHASHI[1], H. OKANO[4];
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Abstract: Pelizaeus-Merzbacher disease (PMD), a form of X-linked leukodystrophy, is caused by proteolipid protein 1 (PLP1) gene mutations. PLP1 proteins with missense mutations are known to accumulate in the rough endoplasmic reticulum (ER) in disease model animals with mutant PLP1 genes. However, its exact pathogenetic mechanism of PMD remains to be clarified. Despite the precise analyses conducted using conventional cellular and animal PMD models, it has not been possible to examine the actual correlation between the known molecular pathogenesis and cell biological phenotypes, including abnormalities in Oligodendrocytes (OLs) differentiation, myelination, and cell death. In addition, those previous results were obtained through analyses using non-human animal models, non-patient-derived cells, or non-oligodendrocyte models, and it is unknown whether these results can be applicable to human patients. Thus, in this study, we focused on the pathologic effects of PLP1 missense mutations and established patients-specific induced pluripotent stem cells (iPSCs) from two PMD patients (PMD1 (PLP1S253T) and PMD2 (PLP1P215S), both of which differ from those in the previously reported PMD animal models. We induced these iPSCs into OL-lineage cells and examined the pathogenic changes in the PMD iPSCs-derived OLs. We confirmed increased apoptosis in PMD iPSCs-derived OLs and the accumulation and mislocalization of mutant PLP1 proteins to the ER. Furthermore, a higher susceptibility to ER stress was observed in PMD

iPSCs-derived OLs than in control upon treatment with two low concentrations of tunicamycin. Collectively, these results suggest that ER stress is involved in the pathogenesis of this disease. In addition, by electron microscopic analysis, we verified decreases in the frequency of myelin formation and the thickness of the myelin sheath compared with control cells. This iPSCs-based disease model reproduces the pathophysiology observed in the CNS of PMD patients, which could provide useful systems for the drug screening for PMD. Moreover, our results demonstrate the usefulness of iPSCs-derived OLs for the analysis of the pathogenic processes of dysmyelinating human neurological and psychiatric disorders. In ongoing study, we attempt application of our current method to regenerative medicine for the damaged CNS using iPSCs-derived oligodendroglial precursor cells in order to induce the re-myelination, which will be discussed in this presentation. We will investigate of novel therapeutic agents for their treatment and the pathogenesis of PMD with PLP1 duplications, which are more common in PMD in the future.

Disclosures: **Y. Numasawa:** None. **Y. Okada:** None. **S. Shibata:** None. **S. Kawabata:** None. **N. Kishi:** None. **W. Akamatsu:** None. **M. Shouji:** None. **A. Nakanishi:** None. **H. Osaka:** None. **K. Inoue:** None. **S. Yamanaka:** None. **K. Kosaki:** None. **M. Nakamura:** None. **T. Takahashi:** None. **H. Okano:** A. Employment/Salary (full or part-time):; M.S. and A.N. are employed by Takeda Pharmaceutical Company Limited. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; the Core Institutes for iPS Cell Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan to H.O.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); None. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents' (e.g., speakers' bureaus); None. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); H.O. has stock and stock option of SanBio, Inc.. F. Consulting Fees (e.g., advisory boards); H.O. is a scientific consultant for SanBio, Inc., Eisai, Co., Ltd., and Daiichi Sankyo, Co., Ltd., S.Y. is a member without salary of the scientific advisory boards of iPierian, iPS Academia Japan, Megakaryon Corporation, and HEALIOS K. K. Japan..

Nanosymposium

382. Disease Modeling Using Pluripotent Stem Cells I

Location: 156

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 382.13

Topic: A.04. Stem Cells

Support: NIH Grant NS048271

NIH Grant HD069184

NIH Grant NS047344

NIH Grant MH087874

NARSAD

MSCRF

SFARI

Title: Modeling a risk factor for schizophrenia in iPSCs and mice reveals defects in adherens junctions and polarity of human and mouse neural stem cell

Authors: *K.-J. YOON[1], H. NGUYEN[2], G.-L. MING[1], H. SONG[1];
[1]Neurol., [2]Grad. Program in Cell. and Mol. Med., Johns Hopkins Univ., Baltimore, MD

Abstract: 15q11.2 copy number variants are prominent risk factors for neuropsychiatric disorders, including schizophrenia and autism. How candidate risk gene(s) within 15q11.2 may regulate brain development is unknown. Here we show that human iPSC-derived neural progenitors carrying 15q11.2 microdeletion exhibit impairments of adherens junctions and apical polarity due to CYFIP1 haploinsufficiency-induced WAVE complex destabilization. In the developing mouse cortex, deficiency in CYFIP1 and WAVE signalling leads to similar defects in radial glia cells, resulting in aberrant positioning of these neural stem cells and their neuronal progeny. In humans, targeted genetic association analyses revealed an epistatic interaction between gene expression-associated variants of CYFIP1 and WAVE signalling mediator ACTR2 to affect risk for schizophrenia. Our multi-faceted approaches identify a critical role and signalling mechanism of CYFIP1 in regulating neural stem cells and provide novel mechanistic insight into how risk factors may contribute to the susceptibility of neuropsychiatric disorders.

Disclosures: **K. Yoon:** None. **H. Nguyen:** None. **G. Ming:** None. **H. Song:** None.

Nanosymposium

382. Disease Modeling Using Pluripotent Stem Cells I

Location: 156

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 382.14

Topic: A.04. Stem Cells

Support: NIH

Title: Use of Human iPS cells to study the neurobiology of Rett syndrome

Authors: *X. TANG[1], J. KIM[1], L. ZHOU[1], L. ZHANG[1], C. CARROMEU[2], A. R. MUOTRI[2], F. H. GAGE[3], G. CHEN[1];
[1]Dept. of Biol., Penn State Univ., State College, PA; [2]Dept. of Pediatrics, Univ. of California San Diego, Sch. of Med., San Diego, CA; [3]Lab. of Genet., The Salk Inst., La Jolla, CA

Abstract: Rett syndrome is a severe form of autism spectrum disorder that is mainly caused by dysfunction of a single gene MeCP2 in the X-chromosome. Previous studies have demonstrated that MeCP2 regulates global gene transcription and have identified a variety of downstream signaling molecules. However, it remains an unanswered question why Rett patients show a developmental regression with a delay in the onset after birth. Here we developed induced pluripotent stem cells (iPSCs)-based human neuron model to study the molecular basis of Rett syndrome. Human iPS cells carrying a disease-relevant MeCP2 nonsense mutation (Q83X) or a missense mutation (N126I) was derived from Rett patients. Human Rett- and corresponding wild-type control neurons were differentiated from iPS cells by a recently developed stem cell/glial cell co-culture system (Tang, et al., 2013). From a series of molecular and functional analyses, we found that human neurons differentiated from iPS cells from Rett patients showed a significant deficit in both GABA and glutamate neurotransmission systems. Importantly, these deficits in GABA and glutamatergic neurotransmission were rescued to levels comparable to wild-type control neurons by treatment with Insulin-like growth factor-1 (IGF-1) or brain-derived neurotrophic factor (BDNF), suggesting that Rett-related phenotypes are reversible, and the synaptic deficits are amendable to therapeutic intervention. We further investigated the underlying molecular mechanism of the GABA and glutamate system deficits using iPS cell-derived human neurons, and confirmed our findings in parallel experiments carried out with mouse neuron cultures. Taken together, we have developed novel human neuronal models of Rett syndrome to understand the neurobiology of the disease, and to develop novel therapeutic treatment for Rett syndrome. This project was supported by grants from NIH and PSU stem cell fund. 1. Tang X., Zhou L., Wagner A. M., Marchetto M. C., Muotri A., Gage F., and Chen G. (2013) Astroglial cells regulate the developmental timeline of human neurons differentiated from induced pluripotent stem cells. Stem Cell Research, 2013, Vol. 11, issue 2. 743-757. (Cover article).

Disclosures: X. Tang: None. **J. Kim:** None. **L. Zhou:** None. **L. Zhang:** None. **C. Carromeu:** None. **A.R. Muotri:** None. **F.H. Gage:** None. **G. Chen:** None.

Nanosymposium

383. Microglia

Location: 144A

Time: Monday, November 17, 2014, 1:00 PM - 3:30 PM

Presentation Number: 383.01

Topic: B.11. Glial Mechanisms

Title: Epigenetic regulation of the JAK/STAT pathway in microglial activation

Authors: *R. PATNALA, T. S. DHEEN;
Anat., Natl. Univ. of Singapore, Singapore, Singapore

Abstract: Microglia cells, the resident immune cells of the brain are known to exhibit diverse beneficial roles in the brain parenchyma. However, these cells upon chronic activation in neuropathological conditions may exacerbate neuroinflammation. Recent studies show members of the Signal Transducer and Activator of transcription (STAT) family to be crucial for microglial activation. STAT proteins play a central role in mediating cytokine signalling through the Janus Kinase (JAK)/STAT pathway. However, no detailed study on the epigenetic regulation of STATs has been carried out in microglia to date. It is hypothesized that epigenetic mechanisms such as chromatin regulation via histone modifications play a vital role in activation of microglia. In this study, we characterized the global expression of histone modification, Histone 3-lysine 9-acetylation (H3K9ac) in microglia. Temporal expression of H3K9ac through immunohistochemistry in microglia in the corpus callosum of 1, 5, 7, 28 days old Wistar rats revealed that this mark is highly expressed in amoeboid microglia and decreases gradually. A recurrence of H3K9ac expression was observed in microglia from 4 weeks old Wistar rats following activation via intraperitoneal injection of lipopolysaccharide (LPS). A similar upregulation of H3K9ac was observed in vitro in LPS-activated BV2 microglia which indicates a role of H3K9ac modulation in activated microglia. At the gene level, chromatin-immunoprecipitation (ChIP) assay revealed a differential H3K9ac enrichment between gene promoters of proinflammatory cytokine TNF-α, and STAT 1, 2, 3, in BV2 microglia, with an increase in H3K9ac enrichment at STAT 3 promoter upon microglial activation. We further showed that histone deacetylases (HDACs) are involved in the maintenance and modulation of H3K9ac enrichment at STAT 1, 2, and 3 gene promoters in microglial activation. We demonstrate that HDAC inhibition (via Sodium butyrate) might be extending its anti-inflammatory action through H3K9ac modulation at the STAT promoters, which might directly influence gene transcription. Thus, HDAC mediated epigenetic regulation of the STAT promoters appears to be involved in regulating the microglial immune response in neuropathological conditions.

Disclosures: **R. Patnala:** None. **T.S. Dheen:** None.

Nanosymposium

383. Microglia

Location: 144A

Time: Monday, November 17, 2014, 1:00 PM - 3:30 PM

Presentation Number: 383.02

Topic: B.11. Glial Mechanisms

Title: Differential release of cytokines by spinal and brain microglia due to glutamatergic injury

Authors: S. BASKAR JESUDASAN[1], M. A. CHURCHWARD[2], K. G. TODD[1], *I. R. WINSHIP[3];
[1]Ctr. for Neurosci., [2]Dept. of Psychiatry, Univ. of Alberta, Edmonton, AB, Canada; [3]Ctr. for Neurosci., Univ. Alberta, Edmonton, AB, Canada

Abstract: Microglia are the primary immune cells of the central nervous system (CNS). Recent studies suggest that microglial responses to perturbations in the CNS are dependent on their immediate environment. For example, our previous work suggests that microglia derived from spinal cord of neonatal rat pups have a reduced inflammatory phenotype in response to endotoxin exposure relative to microglia derived from the brain of the same pups. Insults to the CNS, such as ischemic stroke and spinal cord damage, cause neuronal injury, leading to release of neurotransmitters such as glutamate into the extra cellular milieu. Increases in glutamate concentration in the extra cellular milieu induce excitotoxic injury to neurons and chemotaxis of microglia. However, the influence of immediate environment on microglial release of cytokines in response to glutamatergic injury is not well delineated. Here, we describe a glutamatergic injury model where primary microglia derived from brain or spinal cords were exposed to physiological concentrations of glutamate, mimicking the excitotoxicity that occurs in vivo. Microglial morphology and release of cytokines including interleukin (IL)-1β, IL-6 and tumor necrosis factor alpha (TNF-α) were compared between microglial subpopulations. Interestingly the basal release of TNF- α and IL-1β by brain microglia was higher that of spinal microglia. Acute glutamate treatment increased TNF-α release by both brain and spinal microglia but was markedly higher in microglia derived from brain compared to spinal cord. Differential microglial release of TNF- α and IL-1β after glutamate exposure further supports postulates suggesting that microglia from different CNS regions have different functional characteristics, and these may be important considerations when evaluating immunomodulatory therapies for CNS injury.

Disclosures: **S. Baskar Jesudasan:** None. **I.R. Winship:** None. **K.G. Todd:** None. **M.A. Churchward:** None.

Nanosymposium

383. Microglia

Location: 144A

Time: Monday, November 17, 2014, 1:00 PM - 3:30 PM

Presentation Number: 383.03

Topic: B.11. Glial Mechanisms

Support: Anonymous (CAL, BS)

Title: The glial and stress response to Aβ plaques is altered in C3-deficient APP/PS1 mice

Authors: ***Q. SHI**[1], S. CHOWDHURY[1], R. MA[1], K. LE[1], J. L. FROST[1], J. KENISON[1], S. HONG[2], K. MERRY[3], O. BUTOVSKY[1], K. J. COLODNER[4], B. STEVENS[2], C. A. LEMERE[1]; [1]Ctr. for Neurologic Dis., Brigham and Women's Hospital, Harvard Med. Sch., Boston, MA; [2]F.M. Kirby Neurobio. Ctr., Boston Children's Hosp., Boston, MA; [3]Boston Univ., Boston, MA; [4]5Mount Holyoke Col., South Hadley, MA

Abstract: The complement cascade is an innate immune response to remove pathogens. Complement C3 is elevated in Alzheimer's disease (AD) and Down syndrome brains, colocalizing with neuritic plaques (Stoltzner et al., Am J Pathol 2000), and may contribute to Aβ clearance by microglia (Fu et al., GLIA 2012). Previously, we reported that C3-deficient C56BL/6 mice did not develop age-dependent synapse loss and cognitive decline (Shi et al., SFN 2012 abstract 47.04), and C3-deficiency accelerated Aβ deposition but spared cognitive deficits in aged APP/PS1dE9 Tg mice (Shi et al., SFN 2013 abstract 134.17). How can we explain the increased plaque deposition and protective cognitive effects? Here, we further assessed the effects of C3-deficiency in 16 mo-old male C3-deficient APP/PS1dE9 Tg mice (APP/PS1;C3KO) and APP/PS1dE9 Tg mice by examining Aβ plaque size, plaque-associated gliosis, neuron number, cytokine level and stress-related proteins. We found the following: 1) Aβ42 plaques: C3-deficiency resulted in significantly more large plaques (> 30 mega pixel; 128% increase) and medium plaques (15-30 mega pixel, 56% increase), and less robust increase in small plaques (< 15 mega pixel, 28.7% increase) in the hippocampus of APP/PS1 mice. 2) Gliosis: Although C3-deficiency had no effect on glia cell number, it resulted in decreased association of glia (Iba-1, GFAP) with Aβ plaques in hippocampal CA3 of APP/PS1 mice. Also, we observed increased YM1 (M2) and decreased iNOS (M1) protein levels in APP/PS1;C3KO mice. Plaque-associated resident microglia, detected using microglia-specific 4D4 mAb

developed by Dr. Butovsky's lab, showed ramified phenotype in the hippocampus of C3-deficient APP/PS1 mice, suggesting less activation compared to complement-sufficient APP/PS1 mice. 3) Neurons: We observed significantly more neurons in hippocampal CA3, but not CA1 or DG, of 16 mo APP/PS1;C3KO mice vs. APP/PS1 mice. 4) Cytokines: Pro-inflammatory cytokines were reduced in APP/PS1;C3KO vs. APP/PS1 mice. 5) Stress related proteins: mature-BDNF was significantly elevated in brain homogenates of APP/PS1;C3KO vs. APP/PS1 mice while no difference in pro-BDNF was observed. The number of mineralocorticoid receptor-positive cells was elevated in hippocampal CA3 but not CA1 in APP/PS1;C3KO mice vs. APP/PS1 mice. Our data suggests that lifelong C3-deficiency in APP/PS1 mice protects neurons against age/AD-related changes by reducing the glial response to Aβ plaques, including lowering the secretion of pro-inflammatory cytokines and modulating stress-related proteins, which may protect against cognitive decline.

Disclosures: Q. Shi: None. **S. Chowdhury:** None. **R. Ma:** None. **K. Le:** None. **J.L. Frost:** None. **J. Kenison:** None. **S. Hong:** None. **K. Merry:** None. **O. Butovsky:** None. **K.J. Colodner:** None. **B. Stevens:** None. **C.A. Lemere:** None.

Nanosymposium

383. Microglia

Location: 144A

Time: Monday, November 17, 2014, 1:00 PM - 3:30 PM

Presentation Number: 383.04

Topic: B.11. Glial Mechanisms

Title: Impacts of glutamate and LPS on microglial activation regarding additive neurotoxic effects *in vitro*

Authors: *S. JUNG[1], F. BRACKMANN[2], R. TROLLMANN[2];
[1]Univ. of Erlangen, Erlangen, Germany; [2]Dept. of Pediatrics, Neuropediatrics, Univ. Hosp. Erlangen, Erlangen, Germany

Abstract: Introduction: In addition to its excitatory presynaptic functions, glutamate represents a potent and rapidly acting neurotoxin as shown in various neuronal injury models in vitro and in vivo. Since microglial cells are important mediators of immunological pathways that can easily activated and maintained by endotoxins, here we investigated the neurotoxic impact of glutamate on LPS-stimulated microglia in vitro. Methods: BV2 cells were exposed to LPS (1x105 EU/ml) and glutamate (50nM - 5mM) for 24h - 72h. Expression of the hypoxia inducible transcription factor HIF-1alpha as well as iNOS, IL-1beta, IL-6, and TNF-alpha were analyzed by TaqMan

real-time PCR, Western Blot, and ELISA. NO production was quantified by the Griess assay. Cytotoxicity was determined by trypan blue staining. Results: LPS exposition of BV2 microglial cells prior glutamate stimulation increased mRNA and protein expression of HIF-1alpha (P=0.003), iNOS (P<0.001), IL-1beta (P=0.001), IL-6 (P=0.017), and TNF-alpha (P=0.008) suggesting synergistic regulation of inflammatory mediators by glutamate excitotoxcicity in microglial cells. Whereas HIF-1alpha accumulation was transient depending on the presence of LPS, ongoing cytokine and NO secretion was observed for at least 3d after LPS removal. Notably, glutamate exposition significantly potentiated the LPS-induced secretion of NO by about 800%. Thereby, cell-free supernatants derived from LPS exposed BV2 cells were highly cytotoxic. Again, co-stimulation by increased glutamate concentrations enhanced cytotoxic effects significantly. Conclusion: Present in vitro data indicate that LPS induces a dose-dependent and long-lasting sensitization of BV2 cells to excitotoxic injury. Thereby, subsequent cytokine and NO secretion may promote delayed neurotoxicity meditated by activated microglial cells. Present observation may confirm anti-inflammatory therapeutics as promising neuroprotective options.

Disclosures: S. Jung: None. **F. Brackmann:** None. **R. Trollmann:** None.

Nanosymposium

383. Microglia

Location: 144A

Time: Monday, November 17, 2014, 1:00 PM - 3:30 PM

Presentation Number: 383.05

Topic: B.11. Glial Mechanisms

Title: microRNA-27a targets Smad2, a key mediator of TGF-β signalling in microglia

Authors: *S. JADHAV[1], V. TANAVDE[2], S. THAMEEM DHEEN[1];
[1]Natl. Univ. of Singapore, Singapore, Singapore; [2]A*STAR, Bioinformatics Inst., Singapore, Singapore

Abstract: Microglia, the resident macrophages of the central nervous system (CNS) are activated in response to detrimental signals such as neuronal injury and infection by releasing proinflammatory cytokines and chemokines which attract other immune cells to the site of injury. Microglial activation is the hallmark of neuroinflammatory conditions observed in CNS infections, injury and neurodegenerative diseases. Thus, understanding the mechanism of microglia-mediated inflammation is crucial towards developing neurodegenerative disease therapies. Micro RNAs, a family of small, nonprotein-coding RNAs, control gene expression at

the post-transcriptional level through imperfect base pairing with the 3'UTRs of their target mRNAs. Altered miRNA expression levels have been observed in many types of human diseases, including neuronal disorders. However, limited data is available on the contribution of miRNAs to microglial-mediated immune response. In order to understand the miRNA changes contributing to microglial activation, a global miRNA microarray was carried out using control and activated primary microglia (by LPS or β-amyloid). This screen identified several differentially expressed miRNAs in activated microglia in response to LPS and β-amyloid. Bioinformatic analysis identified miRNAs involved in the TGF-β signalling pathway were upregulated in activated microglia. TGF-β1, an anti-inflammatory cytokine was found to be upregulated in activated microglia in vivo and in vitro whereas TGF-β receptor 1 (TβR1) and smad2, key mediators of TGF-β signalling were found to be downregulated in activated microglia in vitro. miRNA-27a which was identified from the miRNA screen was significantly upregulated in LPS-activated microglia in vitro. Bioinformatic analysis predicted TβR1 and smad2 to be the targets of miRNA-27a. Knockdown of miRNA-27a in BV2 microglia resulted in an increase in TβR1 and smad2 protein expression indicating that these genes are indeed targeted by miRNA-27a in microglia. The function of miRNA-27a in TGF-β signalling pathway in regulating microglial activation will be discussed.

Disclosures: **S. Jadhav:** None. **V. Tanavde:** None. **S. Thameem Dheen:** None.

Nanosymposium

383. Microglia

Location: 144A

Time: Monday, November 17, 2014, 1:00 PM - 3:30 PM

Presentation Number: 383.06

Topic: B.11. Glial Mechanisms

Support: NIH Grant R01NS065052

Phoenix Children's Hospital Mission Support Funds

Title: Morphology alone does not define microglial phenotype

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Abstract: Microglial activation effects neurological function. Solely ramified microglia represent a naïve state; whereas activated microglia, in varying proportions, indicate states of disease. Identifying the markers associated with each microglial morphology would define microglial phenotype. Moreover, manipulation of phenotypes would alter neurological function. Here, we demonstrate that morphologically similar microglia do not necessarily have similar patterns of cytokine reactivity. Adult male Sprague-dawley rats were subjected to midline fluid percussion sham or brain injury to initiate an inflammatory response. Brain tissue was collected at 2h and 6h, 1d, 2d, 7d, 28d and 56d post-injury. Immunohistochemistry for microglia morphology was performed with Iba1 (ionized calcium binding adapter protein). To characterize the phenotype of rod microglia double-labelling was undertaken with Iba1 in conjunction with CD45, CD68 (ED1), or Ox6 (MHCII). Analysis concentrated on the sensorimotor cortex, hippocampus and thalamus. The profile of additional markers including CD11b (Ox42), CD11c (complement 3 receptor), and Isolectin B4 are currently being investigated. Iba1 positive ramified microglia did not show reactivity to CD45, CD68 or Ox6. Following injury-induced microglial activation, Iba1 positive activated, amoeboid and rod microglia were noted. Across the morphologies, not all showed reactivity for CD45. Indeed, activated microglia within the thalamus were highly reactive for CD45; those located in the cortex were not. When amoeboid microglia were present they were reactive for CD45, however rod microglia did not react with this marker. In regards to CD68, no microglial morphology showed reactivity at 2h post-injury. However, by 1d some activated and amoeboid microglia showed reactivity but rod microglia did not. At 7d, in addition to activated and amoeboid microglia, some but not all, rod microglia also showed CD68 reactivity. Although the presence of rod microglia had dissipated at 28d post-injury, CD68 reactivity had increased predominantly in activated and amoeboid microglia. Furthermore, Ox6 was present in some, but not all, activated and rod microglia at 7d. Intriguingly, not all aligned rod microglia reacted to Ox6 despite these cells appearing to be coupled like trains. These data indicate an over simplification in the reliance on morphology for microglial activation state. Studies inevitably need to combine morphology and cytokine receptor levels to more accurately phenotype microglia, for which specific functions have yet to be ascribed.

Disclosures: **J.M. Ziebell:** None. **J. Lifshitz:** None.

Nanosymposium

383. Microglia

Location: 144A

Time: Monday, November 17, 2014, 1:00 PM - 3:30 PM

Presentation Number: 383.07

Topic: B.11. Glial Mechanisms

Support: NIH Grant MH093473

NIH Grant MH097243

NIH Grant AG033028

NIH Grant DE014320

Title: Sympathetic initiation of myeloid cell trafficking from the spleen to the brain caused the re-establishment of anxiety in stress-sensitized mice

Authors: *D. B. MCKIM, J. M. PATTERSON, E. S. WOHLEB, B. JARRETT, J. F. SHERIDAN, J. P. GODBOUT;
The Ohio State Univ., Columbus, OH

Abstract: Repeated social defeat (RSD) in mice recapitulates key immunological, physiological, and behavioral deficits associated with psychosocial stress in humans. We have reported that exposure to sub-threshold stress 24 days after RSD caused re-establishment of anxiety that was dependent on myeloid cell trafficking from the spleen to the brain. Therefore, we hypothesized that the spleen of RSD-exposed mice becomes a critical reservoir of reactive myeloid cells that are rapidly released following sympathetic activation by sub-threshold stressors. In the first experiments, the spleen was removed before 6 cycles of RSD yet this did not alter initial stress-induced myeloid redistribution or anxiety. Splenectomy prior to RSD, however, prevented the re-establishment of myeloid trafficking and anxiety following sub-threshold stress 24 days after RSD. Thus, the spleen did not contribute to myeloid cell trafficking and anxiety immediately following RSD. Rather the spleen served as a reservoir of myeloid cells that were readily released following sub-threshold stress. To address how these cells are released from the spleen, control and RSD-sensitized mice were treated with guanethidine, a peripheral sympathetic inhibitor, 24 days after RSD prior to sub-threshold stress. Intervention with guanethidine prior to sub-threshold stress blocked myeloid cell redistribution and anxiety in RSD-sensitized mice. We interpret these data to mean that sympathetic initiation of myeloid cell trafficking from the spleen to the brain contributes to the re-establishment of anxiety in stress-sensitized mice. Thus, we contend that sympathetic initiation of spleen-to-brain myeloid cell trafficking represents a novel and clinically relevant mechanism in the context of recurring anxiety disorders.

Disclosures: D.B. McKim: None. **J.M. Patterson:** None. **E.S. Wohleb:** None. **B. Jarrett:** None. **J.F. Sheridan:** None. **J.P. Godbout:** None.

Nanosymposium

383. Microglia

Location: 144A

Time: Monday, November 17, 2014, 1:00 PM - 3:30 PM

Presentation Number: 383.08

Topic: B.11. Glial Mechanisms

Support: NIH R01 AG 044404

Title: Phospholipase A2 in Abeta clearance by microglia

Authors: *L. DONG[1], C. B. EST[2], K. B. HENDERSON[2], J. C.-M. LEE[2];
[2]Bioengineering, [1]Univ. of Missouri, Columbia, MO

Abstract: Introduction: Alzheimer's disease (AD) is a neurodegenerative disorder. Accumulation of toxic β-amyloid peptide (Aβ) plays a pivotal role in AD pathology. In fact, imbalance between Aβ production and its clearance has been hypothesized as a factor leading to AD. Although microglia, principle macrophages in brain tissue, are known to remove Aβ, the underlying mechanism has yet to be explained. Cytosolic phospholipase A_2 ($cPLA_2$) and calcium-independent PLA_2 ($iPLA_2$) have been reported to contribute to phagocytic function of monocytes. Since microglia are monocyte-derived cells, we study the role of $cPLA_2$ and $iPLA_2$ in Aβ clearance by microglia. **Materials and Methods:** *Cell culture*- Immortalized mouse microglia cell line, BV-2 cells, were a generous gift from Dr. Gary A. Weisman. *Oligomeric $A\beta_{1-42}$ preparation*- Lyophilized $A\beta_{1-42}$ were purchased from ANASpec, and oligomeric $A\beta_{1-42}$ were performed according to published protocol (Dahlgren et al, 2002). *$A\beta_{1-42}$ ELISA*- Commercial ELISA kit (Invitrogen) was used to measure $A\beta_{1-42}$ uptake. BV-2 cells were incubated with 1 μM $A\beta_{1-42}$ for 1 h. For inhibition groups, cells were incubated with 120 nM BEL (Santa Cruz) or 100 μM MAFP (Santa Cruz) for 30 min before $A\beta_{1-42}$ treatment. Total protein concentrations in cell lysates are determined by a BCA kit (Pierce). ELISA assay of $A\beta_{1-42}$ was performed according to manufacturer's instructions. *Cell viability assessment*- Cell viability was evaluated by MTT assay. *Immunofluorescent imaging*- Lysosomes in BV-2 cells were labelled by Lysosensor Green (Invitrogen) which is a pH-dependent lysosome fluorescent dye. Cells were cultured on cover slips, treated as previously described, and fixed with 4% PFA. Fluorescent imaging was performed by Nikon TE2000-U microscope. *Statistical analysis*- Results were analyzed with one-way ANOVA followed by post hoc Tukey pairwise comparisons. **Results & Discussion:** We examined Aβ uptake by BV-2 cells when $iPLA_2$ or/and $cPLA_2$ activities are blocked by BEL (an inhibitor for $iPLA_2$) or/and MAFP (an inhibitor for both $iPLA_2$ and $cPLA_2$). Data show the amount of Aβ uptake significantly decreased in these inhibition groups. Cell viability of BV-2 cells treated Aβ, BEL or MAFP remained at 80% of the control. Lysosomes in BV-2 cells treated with Aβ also exhibited higher acidity as compared with those without treatment with Aβ, which can be suppressed by inhibitions of $cPLA_2$ and $iPLA_2$. **Conclusions:** We show both $iPLA_2$ and $cPLA_2$ are required for Aβ uptake by BV2 cells. Effects of $iPLA_2$ and $cPLA_2$ on Aβ uptake are

related to lysosome function. Further study will focus on the underlying cytokine signaling pathway of $iPLA_2$ and $cPLA_2$ in lysosomes and the membrane property of microglia.

Disclosures: **L. Dong:** None. **C.B. Est:** None. **K.B. Henderson:** None. **J.C. Lee:** None.

Nanosymposium

383. Microglia

Location: 144A

Time: Monday, November 17, 2014, 1:00 PM - 3:30 PM

Presentation Number: 383.09

Topic: B.11. Glial Mechanisms

Support: National Natural Science Foundation of China (No. 81072242 and No. 81272576)

Fundamental Research Funds for the Central Universities

Funds for Pearl River Science &Technology Star of Guangzhou City (2012J2200088)

Title: Phagocytosis of Microglia in radiation-induced brain injury

Authors: *Y. TANG[1], Z. LI[2], P. XU[2], X. SHI[2];
[1]Neurol., Sun Yat-Sen Mem. Hospital, Sun Yat-Sen Univ., Guangdong, China; [2]Sun Yat-sen Mem. Hospital, Sun Yat-sen Univ., Guangzhou, China

Abstract: Purpose: Microglia plays an important role in radiation-induced brain injury, by secreting inflammatory mediators and phagocytosing cell debris. This research aimed to investigate the role of microglial phagocytosis and the underline signal pathway in radiation-induced brain injury. **Methods:** Male BalB/C mice were used to establish radiation-induced brain injury (RBI) model. Mice were divided into the following four groups: unirradiated controls, unirradiated mice administered with phagocytosis inhibitor (inhibitor control), irradiated group, irradiated mice administered with phagocytosis inhibitor. Irradiation was administered using a 6MV β-ionizing-ray linear accelerator, with a single dose of 30 Gy to the whole brain. Phagocytosis inhibitor, MRS2578 were given daily at dose of 100μmol /L for 7 consecutive days, with first dose starting from 45 minutes prior to irradiation. The morphological changes of microglia were observed and the phagocytic capacity of microglia was analyzed by immunofluorescence. Enzyme-linked immunosorbent assay (ELISA) was used to detect the level of TNF-α and IL-6 after phagocytosis inhibitors were used. In vitro study, primary microglia received a single dose of 8Gy. The RhoA and MLCK protein expression were assayed by Western Blot. **Results:** Radiation led to morphological transformation of microglia, from

branch-like resting state to amoeba-like activated state. The phagocytic capacity showed an increasing trend during 7-30 days after radiation. Phagocytosis inhibitors can effectively decrease the phagocytic ability of microglia in phagocytosing fluorescent microspheres. The number of apoptotic neurons and level of inflammatory cytokines TNF-α, IL-6 were higher in the irradiated mice administered with inhibitor than those in the irradiation group. No significant difference was found between the inhibitor control group and the unirradiated control group. Selective inhibitor of RhoA and MLCK can efficiently inhibit cytoskeleton changes and reduce the phagocytic ability of microglia in phagocytosing fluorescent microspheres. **Conclusion:** Phagocytosis capacity of microglia increased after radiation. By inhibiting phagocytosis, the level of inflammatory cytokines and neuronal apoptosis increased. RhoA and MLCK signaling pathways might involve in regulating phagocytosis of microglia.

Disclosures: Y. Tang: None. **Z. Li:** None. **P. Xu:** None. **X. Shi:** None.

Nanosymposium

383. Microglia

Location: 144A

Time: Monday, November 17, 2014, 1:00 PM - 3:30 PM

Presentation Number: 383.10

Topic: B.11. Glial Mechanisms

Support: NHMRC Grant 1044007

PhD scholarship - Australian Rotary Health, Koo Wee Rup Lang Lang

Title: Microglial phenotype changes and C5aR1 expression in chronic mouse models of epilepsy

Authors: *M. J. BENSON[1], S. MANZANERO[2], K. BORGES[1];
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Abstract: The involvement of inflammation within the CNS as a mechanism for seizure generation and epilepsy development is being investigated. Specifically, we researched microglial phenotypes and complement receptor expression in two chronic epilepsy models after status epilepticus (SE). Measuring hippocampal mRNA levels of microglial activation state markers, we found significant increases of both inflammatory M1 and reparative M2 markers 3 days post pilocarpine-induced SE, compared to NoSE control mice: M1 (IL-1β 3fold *, CD16 3.5fold **, TNFα 11 fold **, CD11b 5 fold **** n=8-12) M2 (Arg1 10 fold *, Ym1 8 fold **,

IL-4 6.3 fold * n=12). However in the chronic phase (21 day post-SE) only some inflammatory mRNA were increased (IL-1β 2.4 fold **, CD11b 7.5 fold * n=12). Similar results were found in the intrahippocampal (i.h.) kainate model. Using flow cytometry, these mRNA changes were only partially confirmed at the protein level in isolated microglia from pilocarpine SE mouse cortices. All mRNA increases seen in the acute phase were also observed in isolated microglial cells, (M1: IL-1β, TNF, IL-6; M2: Ym1, Arg1, IL-4 $p<0.05$, n=6). However, no significant changes of any protein markers were seen in microglia at the chronic timepoint, which is inconsistent with the increased M1 marker mRNA levels found in hippocampus. The lack of inflammatory markers, such as IL-1β, may be due to the fact that other cell types, such as neurons and astrocytes, can express these mRNA and/or protein. Alternatively, transcriptionally the microglia may be in a "primed" inflammatory M1 state in the chronic phase of the disease, but are not "fully" activated. In addition, there was a persistent increase in complement C5a receptor, C5aR1, expression at 3 and 21 days after SE in the pilocarpine model compared to NoSE controls (Hippocampal mRNA: 18 fold ****, 7 fold ** n=12). The number of C5aR1-positive expressing microglia also increased at both timepoints (27 fold *, 1.6 fold * n=6). Similar increases were also seen in i.h. kainate model at the mRNA level at both time points (4 fold *, 1.7 fold, n=12). Analysis of microglial and inflammatory markers in C5aR1- deficient mice is currently under way to determine if C5aR1 can affect the inflammatory microglial phenotype furthering our understanding of the role of C5aR1 in microglia during epileptogenesis. In conclusion our data suggests a change in microglial phenotype during epileptogenesis, namely from a mixed inflammatory and reparative state acutely after SE, to a state lacking reparative markers in the chronic phase. Most likely this contributes to the "epileptic" state, perpetuating a cycle of continued seizures and inflammation.

Disclosures: **M.J. Benson:** None. **S. Manzanero:** None. **K. Borges:** None.

Nanosymposium

384. Synaptic Signaling and Neurotransmitter Deficits in Alzheimer's Disease

Location: 150B

Time: Monday, November 17, 2014, 1:00 PM - 3:30 PM

Presentation Number: 384.01

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

Support: NIH Grant AG000222 (SH)

Coins for Alzheimer's Research Trust (BS)

Anonymous (BS, CAL)

NIH Grant AG006173 (DJS)

Title: Classical complement cascade mediates early synapse loss in Alzheimer's disease mouse models

Authors: *S. HONG[1], K. MERRY[1], S. RAMAKRISHNAN[1], B. NFONOYIM[1], Q. SHI[2], B. A. BARRES[3], C. A. LEMERE[2], D. J. SELKOE[2], B. STEVENS[1];
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Abstract: An early hallmark of Alzheimer's disease (AD) is a progressive, region-specific degeneration of synapses; however, molecular mechanisms that drive synapse loss and dysfunction in AD remain unclear. Synapse loss in the healthy developing nervous system is a normal and highly regulated process required for proper brain wiring and synaptic connectivity. Recent work has identified unexpected roles for the innate immune pathway - proteins of classical complement cascade, C1q and C3, and microglia, immune cells of the CNS - for elimination and refinement of synaptic connections in postnatal mouse brains (Stevens et al., Cell 2007; Schafer et al., Neuron 2012). Interestingly, AD brains have highly increased levels of C1q and downstream complement proteins, and certain complement cascade interactors have emerged as susceptibility genes in AD. Here we hypothesized that mechanisms similar to developmental synapse pruning may be involved to drive synapse loss in the early stages of AD pathogenesis. We found a region-specific upregulation and deposition of complement proteins onto synapses well before cognitive and pathological phenotypes in two mouse models of AD, the J20 hAPP and APP/PS1 transgenic mice. Acute injection of soluble Aβ oligomers into brains of healthy wild type adult mice also induced complement deposition onto hippocampal synapses. Furthermore, genetic deletion of classical complement cascade components protects against early synapse loss and cognitive impairment in AD mouse models. Together, our results suggest that aberrant reactivation of a normal developmental pruning pathway may work together to mediate early synapse loss in pre-plaque brains, marking an important step in development of AD synaptic pathology. This study also has broad therapeutic implications for AD and other age-dependent neurodegenerative diseases involving synaptic loss and dysfunction.

Disclosures: S. Hong: None. **K. Merry:** None. **S. Ramakrishnan:** None. **B. Nfonoyim:** None. **Q. Shi:** None. **B.A. Barres:** None. **C.A. Lemere:** None. **D.J. Selkoe:** None. **B. Stevens:** None.

Nanosymposium

384. Synaptic Signaling and Neurotransmitter Deficits in Alzheimer's Disease

Location: 150B

Time: Monday, November 17, 2014, 1:00 PM - 3:30 PM

Presentation Number: 384.02

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

Title: Glutamate neurotransmission is altered prior to cognitive decline in APP/PS1 mice, a mouse model of Alzheimer's disease

Authors: K. N. HASCUP[1], S. PEHLMAN-REETER[1], *E. R. HASCUP[2];
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Abstract: Substantial indirect evidence supports altered glutamate homeostasis with Alzheimer's disease (AD) as it plays an important role in learning and memory, a main function of the hippocampus. For example, VGLUT1 boutons are elevated in pre-clinical AD cases and APP/PS1 mice (a model of AD), but are significantly reduced and associated with β-amyloid plaque accumulation in end-stage AD cases and APP/PS1 mice, supporting a shift in extracellular glutamate levels during disease progression. However, it is currently not known if, how, or when extracellular glutamate levels and neurotransmission are impacted during the progression of AD. To address this, mice first underwent Morris water maze (MWM) to test cognition during a spatial learning paradigm. Following behavioral testing, we employed *in vivo* electrochemistry to examine glutamate neurotransmission (resting levels, release, and uptake) in the CA1, CA3, and dentate regions of the hippocampus in 2-4 and 6-8 month old male APP/PS1 mice and age-matched C57Bl/6 control mice. Preliminary data support altered glutamate neurotransmission (release and uptake) prior to cognitive decline (no differences were observed in any of the parameters examined in 2-4 month olds) in APP/PS1 mice compared to control mice. Furthermore, these alterations were hippocampal sub-region specific (CA1, CA3, or dentate). For example, we observed a trend of elevated KCl-evoked glutamate release in the CA1 region of 2-4 month APP/PS1 mice compared to age-matched controls (13.9 ± 2.0 μM vs. 7.1 ± 3.7 μM, respectively; n=3). 2-4 month APP/PS1 mice also required significantly less volume of exogenously applied glutamate to achieve the same ~10 μM amplitude as age-matched control mice in the CA1 (21.5 ± 16.5 nl vs. 89.9 ±18.8 nl, respectively; n=3-4; $p<0.05$), but clearance time was unaffected (2.4 ± 0.6 sec vs. 2.5 ± 0.3 sec, respectively; n=3-4). However, we observed no change in KCl-evoked glutamate release in the CA3 region in 2-4 month APP/PS1 mice compared to controls (4.2 ± 1.9 μM vs. 4.3 ± 0.9 μM, respectively; n=3), but there was a trend of increased clearance time in the APP/PS1 mice (3.3 ± 1.5 sec vs. 1.7 ± 0.2 sec, respectively; n=3-4). Although through different mechanisms, an increase in glutamate release in the CA1 and decreased clearance time in the CA3 could both result in elevated resting glutamate levels. Taken together, preliminary data may support the use of glutamate as an early biomarker for AD.

Disclosures: **K.N. Hascup:** None. **S. Pehlman-Reeter:** None. **E.R. Hascup:** None.

Nanosymposium

384. Synaptic Signaling and Neurotransmitter Deficits in Alzheimer's Disease

Location: 150B

Time: Monday, November 17, 2014, 1:00 PM - 3:30 PM

Presentation Number: 384.03

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

Support: NIH R01 AG042513

P01 NS074969

R21 AG045691

Knight Alzheimer's Disease Research Center at Washington University

Title: Temporal relationship between synaptic activity and Abeta generation *in vivo*

Authors: ***J. R. CIRRITO**[1], C. M. YUEDE[1], C. LI[2];
[1]Neurol., Washington Univ., SAINT LOUIS, MO; [2]Biomed. Engin., Florida Intl. Univ., Miami, FL

Abstract: Alzheimer's disease (AD) is initiated by the progressive accumulation of amyloid-beta (Abeta) peptide in the brain as toxic structures such as Abeta oligomers and plaques. Observations in humans show that plaques are found in regions of the brain that display high levels of neuronal activity, sometimes referred to as the default mode network (Buckner et al., 2009). Direct modulation of synaptic activity dynamically regulates brain Abeta levels in awake animals; increased synaptic activity increases brain interstitial fluid (ISF) Abeta levels and vice versa for suppressed activity. These findings strongly suggest a close temporal relationship between synaptic activity and Abeta generation. We have previously used an in vivo microdialysis technique to demonstrate that synaptic activity is temporally linked to ISF Abeta levels. That methodology has limitations of only measuring Abeta every 30-60 minutes; however, Abeta generation likely occurs on the order of seconds to minutes. We have recently adapted an electrochemical technique to study Abeta in vivo on a much faster time scale. The principle behind this approach is that Abeta contains an electroactive tyrosine amino acid at position 10. A voltage applied to the electrode induces oxidation of the tyrosine residue, which releases electrons that the carbon fiber detects as electrical current. We have covalently attached anti-Abeta antibodies to the electrode surface to provide specificity for Abeta detection to the

exclusion of the other proteins and molecules present within the brain extracellular space. In our published studies (Prabhulkar et al., 2012) we show that MIEs containing anti-Abeta antibodies can specifically detect either Abeta40 or Abeta42. In vivo MIE studies show that we can very rapidly, within a minute, detect brain ISF Abeta levels in APP transgenic mice, allowing us to assess fast-acting mechanisms that directly regulate ISF Abeta. Using MIEs in vivo, we are able to detect a rapid increase in ISF Abeta following a rise in synaptic activity. Large increases in synaptic activity raise Abeta levels within minutes in the APP/PS1 mouse brain, highlighting the close temporal relationship between synaptic activity and Abeta generation in the brain. Micro-immunoelectrodes provide a novel way to explore mechanisms of this relationship with very fine temporal resolution.

Disclosures: J.R. Cirrito: None. **C.M. Yuede:** None. **C. Li:** None.

Nanosymposium

384. Synaptic Signaling and Neurotransmitter Deficits in Alzheimer's Disease

Location: 150B

Time: Monday, November 17, 2014, 1:00 PM - 3:30 PM

Presentation Number: 384.04

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

Support: NIH AG030205

ADDF

Title: Structural and functional deficits at hippocampal pre- and postsynaptic sites are reversed by stabilizing calcium in 3xTg-AD mice

Authors: G. E. STUTZMANN[1], C. SCHNEIDER[5], *S. CHAKROBORTY[1], J. WICKS[1], N. KAPECKI[1], D. T. CHRISTIAN[1], S. WIERSEMA[2], D. MAHER[1], C. BRANDON[3], F. SEILER[4], B. VERTEL[3];
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Abstract: Alzheimer’s disease (AD) is increasingly recognized as a synaptic disease in that synaptic deficits underlie the cognitive impairments which characterize AD. Likewise, strategies which preserve synaptic function and structure would effectively maintain memory processes. Fundamental to both synaptic plasticity and dendritic spine structure is calcium signaling. An early indicator of altered calcium homeostasis in human patients and AD mouse models is the

documented increase in ryanodine receptor (RyR; an ER calcium channel) expression. Correspondingly, RyR-mediated calcium release is increased in pre- and postsynaptic compartments in AD mouse models, and may thus initiate synaptic pathophysiology. Here we examine the extent of pathology within presynaptic terminals and post synaptic spines, and establish if stabilizing RyR-calcium signaling is therapeutic. Immunohistochemistry, electron microscopy, 2-photon imaging, electrophysiology, and spine morphological analysis were used in 3xTg-AD and NonTg mice (3-4 months of age) to perform a detailed comparison of hippocampal synaptic structure and function. In the hippocampus of 3xTg-AD mice, there were impairments in both pre- and postsynaptic regions compared to NonTg mice. Structurally, there were fewer intact CA3-CA1 Schaffer collateral synapses in the stratum radiatum, a reduction in the number of stable mushroom spines, and fewer presynaptic vesicles in CA3 terminals. This was coincident with markedly increased synaptically-evoked calcium signals in dendritic spines from CA1 neurons, suppressed short term plasticity expression at CA3-CA1 synapses, and increased frequency of spontaneous vesicle release from CA3 terminals. However, in mice treated with nanocrystal dantrolene, a RyR allosteric modulator, these deficiencies were largely reversed. Synaptic structure and function were restored to NonTg levels, plasticity was returned to normal levels, and aberrant calcium signaling in spines was reversed. Our findings demonstrate that prior to the emergence of detectable memory deficits, there are profound alterations in RyR-calcium signaling within presynaptic terminals and dendritic spines. Preventing this calcium dyshomeostasis is highly effective in preserving hippocampal synapses, and importantly, preventing the loss of stable mushroom spines. In parallel, synaptic plasticity mechanisms are also restored upon RyR stabilization. These data support the mounting evidence that targeting the RyR may be a highly effective therapeutic strategy to preserve synaptic integrity and therefore cognitive function in AD patients.

Disclosures: **G.E. Stutzmann:** None. **C. Schneider:** None. **S. Chakroborty:** None. **J. Wicks:** None. **N. Kapecki:** None. **D.T. Christian:** None. **S. Wiersema:** None. **D. Maher:** None. **C. Brandon:** None. **F. Seiler:** None. **B. Vertel:** None.

Nanosymposium

384. Synaptic Signaling and Neurotransmitter Deficits in Alzheimer's Disease

Location: 150B

Time: Monday, November 17, 2014, 1:00 PM - 3:30 PM

Presentation Number: 384.05

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

Support: NIH AG022550

NIH AG027956

NIH AG010485

NIH RR022570

NIH RR027093

Felix and Carmen Sabates Missouri Endowed Chair in Vision Research

Vision Research Foundation of Kansas City

Title: Loss of spatial memory, learning and motor coordination during normal aging is accompanied by changes in CNS Presenilin 1 and 2 expression levels

Authors: *S. KAJA[1], N. SUMIEN[3], V. V. SHAH[1], I. M. PUTHAWALA[1], A. N. MAYNARD[1], N. KHULLAR[1], A. J. PAYNE[1], M. J. FORSTER[3], P. KOULEN[1,2];
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Abstract: Alzheimer's disease (AD) is the most common form of dementia, affecting more than 5 million Americans and posing a significant burden on the affected individual, caregivers and society. While the more common form of sporadic AD is believed to be of multifactorial origin and no single underlying disease-causing mutation has been identified, a number of genetic loci etiological for the rare familial form of the disease (FAD) have been fund. One of the loci is the group of presenilin (PS) proteins, which are part of the enzymatic core of the γ-secretase complex. Most of the almost 200 identified FAD mutations in PS are located in the gene encoding presenilin-1 (PS1), while presenilin-2 (PS2) mutations are less frequent and typically cause later onset FAD. Herein, we tested the hypothesis that the expression levels of PS proteins may be altered during healthy aging in the absence of pathological PS mutations. We used an established preclinical model for aging to identify associations between PS protein expression and quantitative parameters obtained from behavioral paradigms for spatial memory and learning and motor coordination. We identified significant changes of PS protein expression in both cerebellum, the anatomical locus for motor coordination, and the forebrain, responsible for spatial and motor learning. Specifically, PS1 levels were decreased, while PS2 levels were increased in aged (24 months old) animals, compared with young (6 months old) controls. These changes correlated with the performance in behavioral paradigms for motor coordination and memory and learning, i.e. the bridge walking test and the swim maze test, respectively. Our study presents strong evidence for the differential expression of PS proteins in a non-genetic model for aging, resulting in an overall increase of the PS2 to PS1 ratio. Our findings provide a novel mechanistic basis for a loss in brain function, particularly with respect to spatial memory, learning and motor coordination during normal aging. Given previous studies that had identified an isotype-specific, differential control of brain ryanodine receptor-mediated neuronal Ca^{2+}

signaling by presenilins at the molecular level, the present data resulting from behavioral *in vivo* studies significantly advance this mechanistic concept towards identifying the change in the PS2 to PS1 ratio during brain aging and the resulting changes in intracellular Ca^{2+} signaling as causative for the age-related decline in brain function.

Disclosures: S. Kaja: None. **N. Sumien:** None. **V.V. Shah:** None. **I.M. Puthawala:** None. **A.N. Maynard:** None. **N. Khullar:** None. **A.J. Payne:** None. **M.J. Forster:** None. **P. Koulen:** None.

Nanosymposium

384. Synaptic Signaling and Neurotransmitter Deficits in Alzheimer's Disease

Location: 150B

Time: Monday, November 17, 2014, 1:00 PM - 3:30 PM

Presentation Number: 384.06

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

Support: grant from Università Cattolica

Title: Amyloid-β (Aβ) protein-induced alterations of synaptic function depend on its intraneuronal accumulation

Authors: *C. RIPOLI, S. COCCO, A. MASTRODONATO, D. D. LI PUMA, R. PIACENTINI, F. SCALA, M. D'ASCENZO, C. GRASSI;
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Abstract: Amyloid-β (Aβ) oligomers have been proposed to be key mediators of cognitive decline in Alzheimer's disease (AD). Aβ induces synaptotoxicity irrespective to the genetic predisposition to this pathology. Indeed, brain slices of wild-type mice exposed to nanomolar concentrations of human Aβ showed impaired hippocampal long-term potentiation (LTP). Many studies have proposed that Aβ oligomers exert their synaptotoxic effects by binding to membrane receptors thereby affecting molecular pathways involved in neuronal functions responsible for the transmission and storage of information in the brain. However, to date pharmacological approaches targeting these receptors cannot cure AD or stop its progression. Here, we tested the hypothesis that Aβ42 internalization from the extracellular space, its intraneuronal accumulation (inAβ) and direct interaction with intracellular partners are critical to Aβ42-dependent synaptotoxicity. Basal synaptic transmission was studied in autaptic microcultures and LTP at CA3-CA1 synapses in hippocampal brain slices. Aβ42 injection into the studied neuron produced marked reduction of: i) both AMPA (-62.3±3.1%) and NMDA (-44.2±6.5%)

components of evoked excitatory postsynaptic currents (EPSC); ii) miniature EPSC frequency (-44.9±8.1%) and amplitude (-23.3±9.1%); iii) the ready releasable pool charge (-23.7±8.4%) and its refilling (-49.7±10.4%); iv) LTP (14.9±18.7% vs. 119.7±17.8% with inAβ and vehicle, respectively). Moreover, EPSC amplitudes reached smaller levels after high-frequency synaptic depression together with paired-pulse facilitation. Control experiments performed with the reverse peptide allowed us to exclude that the effects of inAβ on basal synaptic transmission and synaptic plasticity depended on changes in oncotic pressure. To further investigate inAβ synaptotoxicity we used an Aβ variant that does not cross the neuronal plasma membrane and is not uploaded from the extracellular space. This Aβ42 variant had no effects on synaptic transmission and plasticity when applied extracellularly but induced synaptic depression and LTP inhibition after patch-pipette dialysis. Finally, the injection of an antibody raised against human Aβ42 (6E10) in CA1 pyramidal neurons of mouse hippocampal brain slices did not, per se, significantly affect LTP and basal synaptic transmission but it protected against the toxic effects of exAβ42. Collectively, these findings suggest that Aβ42-induced impairment of glutamatergic synaptic function depends on its internalization and intracellular accumulation thus paving the way to a systemic proteomic analysis of intracellular targets/partners of Aβ42.

Disclosures: **C. Ripoli:** None. **S. Cocco:** None. **A. Mastrodonato:** None. **D.D. Li Puma:** None. **R. Piacentini:** None. **F. Scala:** None. **M. D'Ascenzo:** None. **C. Grassi:** None.

Nanosymposium

384. Synaptic Signaling and Neurotransmitter Deficits in Alzheimer's Disease

Location: 150B

Time: Monday, November 17, 2014, 1:00 PM - 3:30 PM

Presentation Number: 384.07

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

Support: NSF IGERT: Video Bioinformatics Grant DGE 0903667

Title: Video analysis of cofilin transport in dendritic spines

Authors: ***A. ZAHEDI**[1], V. ON[2], C. W. COTMAN[4], B. BHANU[2], I. ETHELL[3];
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Abstract: Actin-severing protein cofilin has been implicated in the mechanisms underlying normal synapse plasticity, as well as synapse loss in neurodegenerative disorders such as

Alzheimer's disease (AD). Actin reorganization mediates the morphological restructuring of synapses/postsynaptic spines, and cofilin regulates this process through its ability to sever filamentous actin (F-actin) and to bind G-actin monomers. Cofilin activity is regulated by several known mechanisms. NMDA receptor (NMDAR) activation was shown to activate cofilin through its dephosphorylation involving the calcineurin-Slingshot (SSH)-mediated pathway. NMDAR activation triggers a rapid translocation of wt-cofilin and constitutively active cofilinS3A to dendritic spines, but not the phospho-mimetic cofilinS3D mutant, suggesting that phosphorylation also plays a key role in cofilin trafficking. In this study, we developed a new approach for regulating cofilin activity in dendritic spines using photoactivatable PA-Rac probe in combination with live imaging to track cofilin localization. PA-Rac triggers activation of an opposing Rac1-Pak1-LIM kinase (LIMK1) pathway to suppress the F-actin-severing activity of cofilin through cofilin phosphorylation. Here, we examine cofilin localization in dendritic spines following photoactivation of PA-Rac in 14DIV (days in vitro) hippocampal neurons expressing PA-Rac and wt cofilin, cofilinS3A, or cofilinS3D mutants. Neurons were exposed to 480 nm light to activate PA-Rac and time-lapse imaging was performed to track the localization of GFP-tagged cofilin. Previously, we observed that PA-Rac activation triggered a cyclic export of cofilin from dendritic spines to dendritic shaft. Given this observation, bioinformatics tools were applied to characterize and study cofilin dynamics during PA-Rac photoactivation, by quantitatively assessing its level of local expression and diffusive properties. An algorithm was designed to track the trajectory of cofilin relative to the dendritic spines in control and mutant conditions. Using this software we will further elucidate the temporal and spatial dynamics of cofilin in dendritic spines and underlying mechanisms of cofilin trafficking.

Disclosures: **A. Zahedi:** None. **V. On:** None. **C.W. Cotman:** None. **B. Bhanu:** None. **I. Ethell:** None.

Nanosymposium

384. Synaptic Signaling and Neurotransmitter Deficits in Alzheimer's Disease

Location: 150B

Time: Monday, November 17, 2014, 1:00 PM - 3:30 PM

Presentation Number: 384.08

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

Support: University of Manchester

Title: The study of age-dependent changes in hippocampal network transmission in the triple transgenic Alzheimer's disease mouse model

Authors: *S. H. JEON, J. TURNER;
Univ. of Manchester, Manchester, United Kingdom

Abstract: Alzheimer's disease (AD) is a neurodegenerative condition and form of dementia. Initial cognitive impairment in early AD is due to dysfunction of the hippocampal complex and intracellular β-amyloid (Aβ) is believed to play a major role. To study effect of Aβ over-expression on hippocampal function, the triple-transgenic Alzheimer's disease mouse model (3xTgAD) was used and compared to aged-matched background strain controls. Multi-site recording was performed in vitro on horizontal brain slices using perforated multi-electrode arrays (pMEAs). This allowed the whole hippocampus to be assessed simultaneously for the generation and propagation of spontaneous activity and for the spread locally-evoked synaptic events following electrical stimulation. Input output (I/O) curves and a range of paired-pulse intervals (0.02-1s) were used to measure stimulus-response relationships and short-term plasticity, respectively. The initial area of interest was the CA3 region adjacent to the fimbria, which was evaluated in three different age ranges (3-4, 6-7 and 8-9 months old). This study revealed that, in both control and 3xTgAD mice, there was an age-dependent decrease in both I/O curve slope and paired-pulse facilitation following local cell body layer stimulation in the CA3 region. These results suggested that there was a general decrease in the CA3 associational pathway during aging in both 3xTgAD and control mice. Furthermore, the 3xTgAD mice showed age-dependent changes in excitability in this associational pathway not seen in the controls. High-intensity 2.5V paired-pulse stimulation induced epileptiform activity (EA) in slices from 3xTgAD but not the control mice at 3-4 months. This activity was initiated in the region bordering CA1 and was then propagated both towards CA1 subiculum, and the proximal CA3 region and dentate gyrus. This suggested that by 3-4 months, chronic exposed to Aβ had induced a hyperexcitable network state in the CA3 region of these mice. However, this phenomenon was age-dependent, so that at 6-7 months, the ability to induce this EA was reduced to 50% of the 3xTgAD mice tested, and lost completely at 8-9 months old. These observations suggest that aging and/or a long-term adaptive mechanism then occlude this initial CA3 region hyperexcitablity.

Disclosures: S.H. Jeon: None. **J. Turner:** None.

Nanosymposium

384. Synaptic Signaling and Neurotransmitter Deficits in Alzheimer's Disease

Location: 150B

Time: Monday, November 17, 2014, 1:00 PM - 3:30 PM

Presentation Number: 384.09

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

Support: NIH grant AG042716

Title: α_{2A} adrenergic receptor promotes amyloidogenesis through disrupting APP interaction with SorLA

Authors: *Q. WANG[1], Y. CHEN[1], Y. PENG[1], P. CHE[1], M. GANNON[1], L. LI[3], G. BU[4], T. VAN GROEN[1], K. JIAO[2];
[1]Cell, Developmental and Integrative Biol., [2]Genet., Univ. Alabama, Birmingham, Birmingham, AL; [3]Univ. of Minnesota, Minneapolis, MN; [4]Mayo Clin., Jacksonville, FL

Abstract: Accumulation of amyloid β (Aβ) peptides in the brain is the key pathogenic factor driving Alzheimer's disease (AD). Endocytic sorting of amyloid precursor protein (APP) mediated by the vaculolar protein sorting (Vps10) family of receptors plays a decisive role in controlling the outcome of APP proteolytic processing and Aβ generation. Here we report for the first time that this process is regulated by a G protein-coupled receptor (GPCR), the α_{2A} adrenergic receptor. Genetic deficiency of the α_{2A}AR significantly reduces, whereas stimulation of this receptor enhances, Aβ generation and AD-related pathology. Activation of α_{2A}AR signaling disrupts APP interaction with a Vps10 family receptor, SorLA, in cells and in the mouse brain. Hence, activation of α_{2A}AR reduces Golgi localization of APP and concurrently promotes APP distribution in endosomes and cleavage by β secretase. The α_{2A}AR is a key component of the brain noradrenergic system. Profound noradrenergic dysfunction occurs consistently in patients at the early stage of AD. α_{2A}AR-promoted Aβ generation provides a novel mechanism underlying the connection between noradrenergic dysfunction and AD. Our study also suggests α_{2A}AR as a previously unappreciated therapeutic target for AD. Significantly, pharmacological blockade of the α_{2A}AR by a clinically used antagonist evidently reduces AD-related pathology and ameliorates cognitive deficits in an AD transgenic model, suggesting that repurposing clinical α_2AR antagonists would be an effective therapeutic strategy for AD.

Disclosures: Q. Wang: None. **Y. Chen:** None. **Y. Peng:** None. **P. Che:** None. **M. Gannon:** None. **T. van Groen:** None. **K. Jiao:** None. **L. Li:** None. **G. Bu:** None.

Nanosymposium

384. Synaptic Signaling and Neurotransmitter Deficits in Alzheimer's Disease

Location: 150B

Time: Monday, November 17, 2014, 1:00 PM - 3:30 PM

Presentation Number: 384.10

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

Support: the Astellas Foundation for Research on Metabolic Disorders

a Grant-in-Aid for the Cooperative Research Project from Joint Usage/Research Center(Joint Usage/Research Center for Science-Based Natural Medicine) Institute of Natural Medicine, University of Toyama in 2011

Title: The mechanism of diosgenin-induced cognitive enhancement in Alzheimer's disease model mice and normal mice

Authors: *C. TOHDA[1], X. YANG[1], Y.-A. LEE[2], Y. GOTO[2], I. NEMERE[3];
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Abstract: Our previous study showed that diosgenin, a plant-derived steroidal sapogenin, improved memory and reduced axonal and presynaptic degenerations in an Alzheimer's disease model 5XFAD mice. We also found that diosgenin may work as an exogenous stimulator of $1,25D_3$-MARRS and induces axonal growth and regrowth even in Aβ-induced damaging condition. (reported in SfN Neuroscience 2012). Here, we firstly aimed to clarify functionally important changes in the brain of diosgenin-treated 5XFAD mice. Diosgenin-treated (for 14 days) 5XFAD mice showed memory improvement. Cerebral cortices from wild-type mice, vehicle-treated 5XFAD mice and diosgenin-treated 5XFAD mice were analyzed in 2D-PAGE to elucidate diosgenin-induced changes in protein expressions in the cortex. We focused on several candidate proteins. We secondly aimed to obtain evidence showing that diosgenin facilitates memory ability also in normal mice. Diosgenin treatment in normal mice enhanced object recognition memory and spike firing and cross-correlation in the medial prefrontal cortex and hippocampal CA1. In diosgenin-treated mice, axonal density and c-Fos expression was increased in the medial prefrontal and perirhinal cortices, suggesting that neuronal network activation may be enhanced. The diosgenin-induced memory enhancement and axonal growth were completely inhibited by i.c.v. injection of a neutralizing antibody for $1,25D_3$-MARRS. Our *in vivo* data indicate that diosgenin is a memory-enhancing drug and that enhancement by diosgenin is mediated by $1,25D_3$-MARRS-triggered axonal growth.

Disclosures: C. Tohda: None. **Y. Lee:** None. **Y. Goto:** None. **I. Nemere:** None. **X. Yang:** None.

Nanosymposium

385. Neuroimaging in Alzheimer's Disease and Tauopathies

Location: 152B

Time: Monday, November 17, 2014, 1:00 PM - 3:00 PM

Presentation Number: 385.01

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

Support: Grants-in-Aid for Japan Advanced Molecular Imaging Program and Science Research

NIH/NINDS P50 NS072187

Title: Visualization of tau lesions in tauopathy brains using tau ligand PBB3

Authors: *N. SAHARA[1], M. ONO[1], S. KOGA[2], J. MAEDA[1], I. MATSUMOTO[1], D. W. DICKSON[2], Z. K. WSZOLEK[2], M.-R. ZHANG[1], T. SUHARA[1], M. HIGUCHI[1];
[1]Mol. Imaging Ctr., Natl. Inst. of Radiological Sci., Chiba, Japan; [2]Mayo Clin., Jacksonville, FL

Abstract: Association of tau deposition with neurodegeneration in Alzheimer's disease (AD) and related tau-positive neurological disorders collectively referred to as tauopathies indicates contribution of tau aggregates to neurotoxicity. The discovery of tau gene mutations in FTDP-17-*tau* kindreds has provided unequivocal evidence that tau abnormalities alone can induce neurodegenerative disorders. Therefore, visualization of tau accumulation would offer a reliable, objective index to aid diagnosis of tauopathy and to assess the disease progression. Positron emission tomography (PET) imaging of tau lesions is currently available using several tau PET ligands including [^{11}C]PBB3. PBB3 is a fluorescent compound, and is accordingly supposed to be useful for multimodal imaging studies, potentially allowing us to validate its binding to diverse tau inclusions by histological methods. In this study, we examined pathological characteristics of tau lesions in brains of human subjects with various tauopathies and tau transgenic mice. Double labeling of brain sections with tau oligomer specific antibody TOC1 and PBB3 demonstrated incorporation of tau oligomers in PBB3-positive tau lesions in old tau transgenic mice named rTg4510 mice, but the appearance of TOC1-positive tau inclusions preceded PBB3 signals. The numbers of TOC1- and PBB3-positive inclusions increased with aging in rTg4510 mice, while most PBB3-positive tau lesions in human AD brains, including ghost tangles, were not labeled with TOC1 antibody. These observations suggest that PBB3 reacts with mature tangles but not tau oligomers, and that tau oligomers in model mice and AD patients are distinctly involved in the formation of tau aggregates. Further in vitro histological/autoradiographic and in vivo PET studies are ongoing to validate the binding of PBB3 to tau lesions in AD and non-AD tauopathies.

Disclosures: **N. Sahara:** None. **M. Ono:** None. **S. Koga:** None. **J. Maeda:** None. **I. Matsumoto:** None. **D.W. Dickson:** None. **Z.K. Wszolek:** None. **M. Zhang:** None. **T. Suhara:** None. **M. Higuchi:** None.

Nanosymposium

385. Neuroimaging in Alzheimer's Disease and Tauopathies

Location: 152B

Time: Monday, November 17, 2014, 1:00 PM - 3:00 PM

Presentation Number: 385.02

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

Support: NIH 5R37AG006265

Title: Aberrant fMRI activity in the medial temporal lobe is related to cortical amyloid-beta deposition in cognitively normal elderly

Authors: *Z. SONG, D. C. PARK;
Ctr. for Vital Longevity, Univ. of Texas at Dallas, Dallas, TX

Abstract: Cortical amyloid-beta (Aβ) deposition in normal aging is a major risk factor of Alzheimer's disease. The pathological relationship remains unclear between cortical Aβ deposition and tau accumulation in the medial temporal lobe, the other hallmark pathology of Alzheimer's disease. In the present study we test the hypothesis that cortical Aβ deposition is related to neural activity in the medial temporal lobe and that this may be an important harbinger of the onset of dementia. Cognitively normal elderly participants (N=63, aged 60 - 90 years) from the Dallas Lifespan Brain Study had brain Aβ deposition assessed by florbetapir F 18 positron emission tomography. All participants underwent fMRI scan during an incidental memory encoding task of viewing a series of natural scene pictures. Task-related brain regions were identified by an independent group of cognitively normal young adults and were used as an inclusive mask. In the elderly group, we examined the relationship between brain Aβ load and task-related activity for which age, gender, and education years were taken into account. In an analysis restricted to the medial temporal lobe, two cluster regions were found in the right hemisphere where task-related activity was reduced with increasing brain Aβ load: one was located in parahippocampal cortex and the other was located at the border of anterior hippocampus, perirhinal cortex, and entorhinal cortex. In a whole brain analysis, similar relationship between brain Aβ load and task-related activity was found in cuneus and posterior cingulate cortex. These findings indicate that cortical Aβ deposition is associated with reduction of functional specificity in the medial temporal lobe memory system together with other brain areas.

Disclosures: Z. Song: None. **D.C. Park:** None.

Nanosymposium

385. Neuroimaging in Alzheimer's Disease and Tauopathies

Location: 152B

Time: Monday, November 17, 2014, 1:00 PM - 3:00 PM

Presentation Number: 385.03

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

Support: NIH Grant AG034570

Fulbright Scholarship

Title: Brain correlates of subjective cognitive complaint differ between healthy elderly individuals with and without β-amyloid pathology

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Abstract: Subjective cognitive complaint (SCC) may be one of the earliest symptomatic manifestations of Alzheimer's Disease (AD). In this cross-sectional study, we explore the relationship between SCC and markers of brain structure and function, and whether these relationships differ in the presence of beta-amyloid (Aβ) plaques. A subjective cognitive complaint measure (GDS-SCC) was generated from the Geriatric Depression Scale (GDS) using principle axis factor analysis on data from 347 healthy, non-depressed (GDS < 11) elderly individuals from the Berkeley Aging Cohort Study. Structural magnetic resonance imaging (MRI) and resting ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) scans were acquired from a subsample (n = 112, mean age 76, 56% female). Pittsburgh Compound B (PiB)-PET scans were acquired to determine the presence (PiB+) or absence (PiB-) of Aβ pathology. Whole-brain voxelwise approaches were used to assess the relationship between glucose metabolism (measured with FDG-PET) and GDS-SCC. Hippocampal volumes (HV) were obtained from the MRI using Freesurfer. Relationships between GDS-SCC and other variables were assessed across the whole sample, as well as stratified by PiB status. GDS-SCC was correlated with other measures of cognitive self-appraisal ($p<0.05$). GDS-SCC predicted global cognition across the whole sample ($p<0.05$), but predicted episodic memory only in PiB+ subjects ($p<0.05$). More cognitive complaints were related to lower glucose metabolism in bilateral caudate nuclei and subgenual anterior cingulate cortex ($p<0.005$ uncorrected, $k > 800$), regions associated with affect and depression. These results were replicated in a separate sample of 334 cognitively normal elderly adults from the Alzheimer's Disease Neuroimaging Initiative.

(However, in both samples, the relationship between metabolism in these regions and GDS-SCC was present only in PiB- individuals. FDG in the SCC-ROI predicted episodic memory and HV across the whole sample, but HV was related to memory in PiB+ subjects only. We show SCC to be associated with variance in brain structure and function in healthy elderly. We suggest that this relationship occurs through two distinct pathways according to Aβ status. Complaints in PiB+ subjects may be due to Aβ-dependent changes to hippocampal structure causing a valid perceived decline in memory. In PiB- individuals, complaint may be driven by subsyndromal affective factors, evidenced by metabolic dysfunction in a cortico-striatal network crucial to emotional encoding and integration. The accurate assessment of memory performance by PiB+ individuals supports SCC as an important feature of preclinical AD.

Disclosures: J.W. Vogel: None. **M. Varga Dolezalova:** None. **R. La Joie:** None. **S.M. Landau:** None. **A. Fero:** None. **S.M. Marks:** None. **H.D. Schwimmer:** None. **W.J. Jagust:** None.

Nanosymposium

385. Neuroimaging in Alzheimer's Disease and Tauopathies

Location: 152B

Time: Monday, November 17, 2014, 1:00 PM - 3:00 PM

Presentation Number: 385.04

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

Support: NIH/NIA Grant 5R01 AG021927

UAB Department of Neurology

Title: A neuroimaging model for loss of financial capacity in Alzheimer’s disease

Authors: *D. L. KERR[1], T. A. BARTEL[1], D. G. MCLAREN[2], D. C. MARSON[1];
[1]Neurol., The Univ. of Alabama At Birmingham, Birmingham, AL; [2]Neurol., Massachusetts Gen. Hosp. and Harvard Med. Sch., Charlestown, MA

Abstract: INTRODUCTION: Neuroimaging can link functional brain networks, brain macro and microstructure, and cognition to understand loss of financial capacity (FC) in Alzheimer’s disease (AD). We developed a neuroimaging model of FC by integrating structural MRI (sMRI) and resting state fMRI connectivity (rsfMRI) relationships with FC in cognitively normal elderly (CN), patients with mild cognitive impairment due to AD (MCI), and patients with mild dementia due to AD (AD). METHODS: Participants were 44 CN, 23 MCI, and 24 AD (sMRI);

and 51 CN, 27 MCI, and 31 AD (rsfMRI) diagnosed in consensus conference. Participants completed the Financial Capacity Instrument (FCI), cognitive assessments, and structural and resting state fMRI scans. For sMRI, SPM8 with DARTEL was utilized to estimate participants' local grey matter volume (GMV). Two-sample t-tests assessed GMV differences between groups. The relationship of GMV with FCI score was assessed with linear regression models. Both analyses included age, education and total brain volume as covariates. We report clusters with $p<0.05$ corrected for multiple comparisons. For rsfMRI, time series data was extracted from four seed regions: (1) the posterior cingulate cortex, (2) medial prefrontal cortex, and (3/4) left/right inferior parietal lobules. Seed region time courses were correlated with whole brain. Correlations were normalized using Fisher's r-to-z transform. Two-sample t-tests assessed connectivity differences between groups. The relationship of connectivity with FCI score was assessed using linear regression models. Both analyses included age and education as covariates. Results were thresholded at $p<0.01$ in at least 10 voxels. RESULTS: Significant differences in FCI score existed across groups ($p<0.001$). We found that (1) GMV decreased from CN to MCI to AD, (2) DMN connectivity decreases in the MCI and AD groups relative to CN, (3) temporal and parietal/occipital cortical atrophy was associated with impaired FCI performance in patients with MCI and mild AD, respectively; and (4) connectivity within the DMN, and between DMN and other brain networks, was related to FCI score. CONCLUSIONS: Both sMRI and rsfMRI provide key insights into the neural basis of FC decline in AD. Specifically, sMRI provides evidence of how cortical atrophy in AD is associated with FC loss, while rsfMRI provides evidence of how functional integrity of brain networks, in regions vulnerable to AD pathology, may mediate FC-structure relationships. Taken together, these two imaging approaches can help explain the neural basis of the loss of financial capacity in AD, which is often accompanied by financial exploitation.

Disclosures: D.L. Kerr: None. **T.A. Bartel:** None. **D.C. Marson:** None. **D.G. McLaren:** None.

Nanosymposium

385. Neuroimaging in Alzheimer's Disease and Tauopathies

Location: 152B

Time: Monday, November 17, 2014, 1:00 PM - 3:00 PM

Presentation Number: 385.05

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

Support: DBT (Govt of India)

Title: Brain glutathione depletion in Alzheimer's disease: A magnetic resonance spectroscopic study

Authors: *P. K. MANDAL[1,2], S. SAHARAN[3], G. MURARI[3], M. TRIPATHI[4];
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Abstract: Accumulating evidence suggests oxidative stress (OS) to be a major determinant of Alzheimer's disease (AD) onset and its ineluctable progression. Elevated oxidative damage in AD has been postulated to be a consequence of altered levels of antioxidant enzymes in the brain. The most abundant brain antioxidant is the reduced from of glutathione (GSH), which plays a very important role in maintaining redox homeostasis in the brain. Several lines of evidence from both vivo animal models of AD and in post mortem neurochemical studies suggest that impaired GSH metabolism has substantial importance in the pathogenesis of AD. As such, we conjectured that 1) there would be AD-associated regional reductions in brain GSH levels, and that 2) GSH estimation in these regions would be useful for differential diagnosis of patients with AD from healthy old (HO) individuals as well as from patients with the prodrome to AD, i.e. mild cognitive impairment (MCI). We used proton 1H MRS with specialized MEGA-PRESS sequence to measure in vivo GSH levels of HO, MCI, and AD subjects in key brain regions affected by AD pathology i.e. bilateral hippocampi (Hp) and frontal cortices (Fc). We showed that AD patients have significantly decreased GSH levels in both the left (LH; p=0.000) and right Hp (RH, p=0.01) as well as the right Fc (RFC; p=0.006) when compared to HO as well as MCI subjects. In contrast, no significant difference was observed across subject groups in GSH levels in the cerebellum, a region spared in AD pathology, thus indicating that GSH levels are modulated in a region-specific manner in AD. Further, we found significant positive correlation between cognitive function and GSH levels in the LH and RFC regions. Odds ratios estimation showed that decrease in GSH levels of RFC and LH in HO was associated with 3.56 and 2.26 fold increase, respectively, in risk of developing AD. Using receiver operating characteristics (ROC) analysis, we showed that the combined estimation of GSH in RFC and LH yielded the highest diagnostic accuracy of 90.3% with 85.7% sensitivity and 94.1% specificity in differentiating AD from HO. Further, GSH levels in RFC and LH also reliably differentiated patients with AD from those with MCI with a significant accuracy of 85.2% with 78.6% sensitivity and 92.3% specificity. The present study offers novel insight into the molecular underpinnings of AD pathology and convincingly evidences that GSH levels are selectively reduced in Fc and Hp brain regions in AD. The results of our ROC analysis are strongly indicative of the potential of GSH estimation as a diagnostic biomarker for AD. * Corresponding Author Dr. Pravat k Mandal

Disclosures: **P.K. Mandal:** None. **S. Saharan:** None. **G. Murari:** None. **M. Tripathi:** None.

Nanosymposium

385. Neuroimaging in Alzheimer's Disease and Tauopathies

Location: 152B

Time: Monday, November 17, 2014, 1:00 PM - 3:00 PM

Presentation Number: 385.06

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

Support: General Electric Company Sponsor ID A110451.01

Title: Imaging neuroinflammation in mice using the 18F-GE180 TSPO PET tracer

Authors: *B. LIU[1], K. X. LE[1], M.-A. PARK[2], S. WANG[2], A. P. BELANGER[2], S. DUBEY[2], P. HOLTON[2], V. REISER[3], P. JONES[3], W. TRIGG[3], M. F. DI CARLI[2], C. A. LEMERE[1]; [1]Dept. of Neurol., Ctr. for Neurologic Diseases, Brigham & Women's Hosp. and Harvard Med. Sch., Boston, MA; [2]Radiology, Dept. of Radiology, Brigham & Women's Hosp. and Harvard Med. Sch., Boston, MA; [3]GE Healthcare, Amersham, United Kingdom

Abstract: Alzheimer's disease (AD) is the most common cause of dementia in the elderly. Neuroinflammation is thought to play an early and important role in AD pathogenesis. The ability to detect microglial activation *in vivo* in patients may allow for selective monitoring of the progression of neuroinflammation as well as to assess efficacy in therapeutic trials. The 18 KDa translocator protein (TSPO), a marker for activated microglia, has been used as a positron emission tomography (PET) tracer target to reflect cerebral inflammation *in vivo* in human and transgenic (Tg) mouse models. In this study, we utilized the ^{18}F-labeled GE180 PET tracer, a new TSPO ligand, to investigate the differences in neuroinflammation between young wildtype (Wt, 4 mo-old, n=4), old Wt (26 mo-old, n=4), and old AD Tg mice (26 mo-old, n=4). *In vivo* PET imaging revealed an overt age-dependent elevation in whole brain uptake of ^{18}F-GE180 (peak-uptake and retention) in Wt mice from 4 mo to 26 mo of age, and a significant increase in whole brain uptake of ^{18}F-GE180 in old APP/PS1 mice compared to either old or young Wt mice. A similar result was observed in hippocampal-specific uptake of ^{18}F-GE180 (old Tg > old Wt > young Wt) using co-registration of PET images with mouse brain MRI, suggesting that both aging and AD pathogenesis result in an increase in neuroinflammation. Quantitative analysis of ^{18}F-GE180 binding in cortex and hippocampus in 1 mm mouse brain slices by *ex vivo* PET and autoradiography (AR) further confirmed the *in vivo* PET results. The $SUV_{75\%}$ was determined to define the specificity of ^{18}F-GE180 uptake in the cortex and hippocampus, and revealed that old Wt mice had significantly enhanced uptake and specific binding of ^{18}F-GE180 compared to young Wt mice, and that old APP/PS1 Tg mice had an even higher uptake and binding compared to all Wt mice. The specificity of ^{18}F-GE180 uptake in the brain was further confirmed by our *in vivo* cold tracer competition study, in which ^{18}F-GE180 labeling was

dramatically reduced by pre-injection with unlabeled ("cold") GE180. In addition, we examined GE180 metabolites in 4-month-old Wt mice and found that even though total radioactivity declined over 2 hours, of the remaining radioactivity, ~90% was parent GE180. Taken together, our studies indicate that GE180 has potential as a novel PET tracer for neuroinflammation and may be useful for diagnosis, disease progression and monitoring treatment effects in human neurodegenerative diseases and animal models.

Disclosures: B. Liu: None. **K.X. Le:** None. **M. Park:** None. **S. Wang:** None. **A.P. Belanger:** None. **S. Dubey:** None. **P. Holton:** None. **V. Reiser:** None. **P. Jones:** None. **W. Trigg:** None. **M.F. Di Carli:** None. **C.A. Lemere:** 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.; GE Healthcare.

Nanosymposium

385. Neuroimaging in Alzheimer's Disease and Tauopathies

Location: 152B

Time: Monday, November 17, 2014, 1:00 PM - 3:00 PM

Presentation Number: 385.07

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

Support: R-37-AG06265

IIRG-09-135-087

Avid Radiopharmaceuticals provided the radiotracer for this project

Title: Structural consequences of amyloid burden across the adult lifespan

Authors: *G. N. BISCHOF[1], K. M. KENNEDY[1], I. MCDONOUGH[1], K. M. RODRIGUE[1], J. R. RIECK[1], M. D. DEVOUS, SR[2], D. C. PARK[1];
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Abstract: Gray matter atrophy is a fundamental characteristic of neurodegeneration in Alzheimer's disease (AD). Specific cortical thinning patterns are indicative of AD status and the progression from prodromal stages to the clinical diagnosis of probable AD. In addition to cortical thinning, AD patients also exhibit elevated levels of fibrilliar amyloid (beta-amyloid). However, unlike cortical thinning, beta-amyloid is an early biomarker and plays a critical role in

initiating a cascade of pathophysiological events that ultimately leads to AD. Using Positron Emission Tomography (PET), beta-amyloid has been observed in vivo in clinically normal older adults above the ages of 60, but the direct or indirect effect of beta-amyloid burden on cortical brain aging and its relationship with progression to AD is still under debate. In fact some research has shown both increases and decreases in thickness associated with beta-amyloid pathology. Here we examined the impact of beta-amyloid burden on whole brain thickness from a healthy aging perspective by including healthy older adults across the adult lifespan, ranging in age from 30 to 89 years (N=142, Mage= 63.52; SDage= 16.68) who underwent structural imaging and PET to quantify beta-amyloid burden using 18F Florbetapir as an imaging ligand. Cortical amyloid was computed by extracting counts across 8 cortical regions of interest, normalized to cerebellar gray matter (SUVR). Participants were categorized into amyloid negative and amyloid positive individuals based on a median split on SUVR values. Cortical scans were processed using FreeSurfer, and amyloid status and continuous age were entered in a general linear model, controlling for the effect of apolipoprotein E. We observed an age × amyloid status interaction in bilateral posterior regions including occipital cortex, precuneus and parietal cortices as well as left lateralized frontal regions. These interactions were characterized by steeper age-related thinning patterns in amyloid positive individuals than amyloid negative individuals. Furthermore, elevated beta-amyloid was associated with greater thickness in middle aged adults (i.e., 30-49 years), but less thickness in older adults (i.e., 70-89). These results make an important contribution to our understanding of the consequences of AD pathology across the adult lifespan. That is, in younger ages, high amyloid deposition may lead to a compensatory response in the form of molecular or physiologic mechanisms providing protection against AD pathology, while in older ages, these mechanisms may not be sufficient enough to sustain the impending effect of amyloid deposition.

Disclosures: G.N. Bischof: None. **K.M. Kennedy:** None. **I. McDonough:** None. **K.M. Rodrigue:** None. **J.R. Rieck:** None. **M.D. Devous, Sr:** None. **D.C. Park:** None.

Nanosymposium

385. Neuroimaging in Alzheimer's Disease and Tauopathies

Location: 152B

Time: Monday, November 17, 2014, 1:00 PM - 3:00 PM

Presentation Number: 385.08

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

Title: Diffusion tensor imaging detects widespread white matter alterations in pericyte-deficient mice

Authors: *M. DAIANU[1], N. JAHANSHAD[2], R. JACOBS[4], B. V. ZLOKOVIC[3], A. MONTAGNE[3], P. M. THOMPSON[2];
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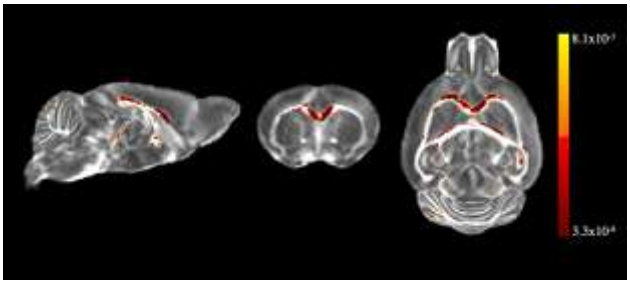
Abstract: White matter disorders, including degenerative brain diseases, can be monitored with sensitive neuroimaging methods, such as diffusion-weighted imaging (DWI), to assess the integrity of the brain's neural pathways. More recently, it has been hypothesized that diseases of the aging brain might involve a blood-brain barrier breakdown caused by a reduction in pericyte induced microcirculation. Here, we developed and tested a novel protocol to process high-field DWIs in pericyte-deficient mice, and to test for white matter alterations relative to healthy wild-types. Briefly, we processed 21 mice - 10 pericyte-deficient and 11 wild-types - all scanned post-mortem (at 3-5 months and 9-12 months) to acquire 7 separate images for each DWI sequence using an 11.7 T Bruker BioSpin MRI scanner. After correcting for eddy current distortions and removing extracerebral tissue in each scan, we linearly and elastically registered all images to ensure that all scans were in the same space. Then, fractional anisotropy (FA) was computed and a Gaussian lowpass filter of size 3 was applied to the FA maps. To test for group differences between healthy and diseased mice, we ran a voxel-wise linear regression, controlling for age, and used regional FDR to correct for multiple comparisons. We found a significant and widespread decrease in FA in the pericyte-deficient mice, relative to wild-types (FDR p-value=8.1x10-3) in their corpus callosum, cingulum, internal and external capsule and cerebellum (see figure). Similar findings were previously detected using histological assessments of the same pericyte-deficient mice. Our results indicate that DWI may be highly sensitive to detecting white matter alterations, typically only detectable with ground-truth histological studies. This work is in preparation for larger scale brain connectivity analyses to better understand images obtained in clinical studies of humans. These efforts will provide clinicians and researchers with a much-needed insight into the neurobiological mechanisms involved in disease progression assessed with in vivo imaging.



Disclosures: **M. Daianu:** None. **N. Jahanshad:** None. **R. Jacobs:** None. **B.V. Zlokovic:** None. **A. Montagne:** None. **P.M. Thompson:** None.

Nanosymposium

386. Neurodegeneration Mechanisms in Parkinson's Disease

Location: 147A

Time: Monday, November 17, 2014, 1:00 PM - 4:45 PM

Presentation Number: 386.01

Topic: C.03. Parkinson’s Disease

Support: NIH Grant 5RO1NS06493404

NIH Grant 5F31NS08196302

Title: Pathogenic mutations in LRRK2 enhance pro-inflammatory responses

Authors: *M. S. MOEHLE, J. P. L. DAHER, A. B. WEST;
Neurol., Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Rare missense mutations in the Leucine-Rich Repeat Kinase 2 (LRRK2) gene lead to late-onset Parkinson’s disease (PD), and common genetic variation in LRRK2 is linked to PD, Crohn’s, and Hansen’s disease. The identification of high-specificity LRRK2 monoclonal antibodies has resulted in the discovery that LRRK2 expression is very high in myeloid cells (macrophages, monocytes, etc) of the peripheral immune system. LRRK2 expression becomes further induced in these cells in response to pro-inflammatory stimuli including IFN-gamma, suggesting a possible function for LRRK2 in inflammatory cell signaling and/or myeloid cell differentiation. In past studies, we utilized shRNA knockdown and non-selective LRRK2 inhibitors to observe attenuation of pro-inflammatory responses to stimulation from lipopolysaccharide exposures. Here, we isolate peripheral macrophage cells from carefully characterized G2019S-BAC and knockout rats and mice to study the role of LRRK2 and LRRK2 kinase activity in pro-inflammatory responses. We characterize the LRRK2 interactome in these cells, as well as global assays using RNAseq and measurement of the phospho-proteome to observe how macrophages depend on LRRK2 expression and LRRK2 kinase activity for a full response to pro-inflammatory stimuli. Our data indicate that mutations in LRRK2 cause a general increase in inflammatory signaling and pro-inflammatory cytokine release. This suggests that LRRK2 acts as an important modulator of the inflammatory state in inflammatory cells, and may be an important therapeutic target to treat a number of inflammatory diseases where chronic macrophage activity underlies aspects of dysfunction.

Disclosures: **M.S. Moehle:** None. **J.P.L. Daher:** None. **A.B. West:** None.

Nanosymposium

386. Neurodegeneration Mechanisms in Parkinson's Disease

Location: 147A

Time: Monday, November 17, 2014, 1:00 PM - 4:45 PM

Presentation Number: 386.02

Topic: C.03. Parkinson’s Disease

Title: LRRK2 kinase activity modulates neuroinflammation in microglia cells

Authors: *I. RUSSO, G. BERNARDO, L. CIVIERO, L. BUBACCO, E. GREGGIO;
Dept. of Biol., Univ. of Padova, Padova, Italy

Abstract: Growing evidence indicates that neuroinflammation can contribute to dopaminergic neuron degeneration and progression of Parkinson’s disease (PD). Remarkably, current literature highlights that Leucine-rich repeat kinase 2 (LRRK2), a kinase mutated in autosomal-dominantly inherited and sporadic PD cases, is highly expressed in immune cells, where it appears to regulate inflammation in response to pathological stimuli through a yet undisclosed mechanism. In this study, using GSK2578215A (GSK), a potent and selective LRRK2 inhibitor, we demonstrated that LRRK2 kinase activity controls the induction of inflammatory mediators after LPS-mediated inflammation in BV2 microglia cells. In support of this, primary microglia cells from LRRK2 knockout mice showed a marked reduction in inflammatory cytokines after LPS treatment compared to wild-type microglia, further supporting the involvement of LRRK2 in neuroinflammation. Moreover, we observed that LPS induced a marked phosphorylation of LRRK2 at serine 935 within 3 hours, whereas treatment with GSK reduced both LRRK2 phosphorylation and the related induction of inflammatory mediators upon LPS priming. In conclusion, our results provide robust evidence that LRRK2 plays a crucial role in inflammatory processes. We are currently investigating which molecular pathways LRRK2 impacts, to understand whether LRRK2 controls the transcription or the translation of inflammatory genes in microglia cells after an inflammatory stimulus.

Disclosures: I. Russo: None. **G. Bernardo:** None. **L. Civiero:** None. **L. Bubacco:** None. **E. Greggio:** None.

Nanosymposium

386. Neurodegeneration Mechanisms in Parkinson's Disease

Location: 147A

Time: Monday, November 17, 2014, 1:00 PM - 4:45 PM

Presentation Number: 386.03

Topic: C.03. Parkinson's Disease

Support: Michael J Fox Foundation grant CES FPM

Title: Alpha synuclein loss-of-function toxicity can be rescued by a non-aggregatable form of the protein

Authors: ***M. J. BENSKEY**[1,2], C. JIANG[3], C. E. SORTWELL[2], N. KANAAN[2], F. P. MANFREDSSON[2];
[1]Michigan State Univ., EAST LANSING, MI; [2]Translational Sci. and Mol. Med., Michigan State Univ., Grand Rapids, MI; [3]Hlth. Sci. Ctr., Univ. of Texas, Houston, TX

Abstract: Parkinson Disease (PD) is characterized by loss of dopamine (DA) neurons in the substantia nigra pars compacta (SNpc) and protein inclusions known as Lewy bodies. Aggregated alpha-synuclein (α-syn) is a main component of Lewy bodies and mutations or multiplications of the α-syn gene result in familial PD. The correlation between abnormal α-syn and cell loss in PD has led to the theory that α-syn-mediated pathology arises due to a toxic gain-of-function, and current therapeutic strategies center on eliminating α-syn from DA neurons. However, we have shown that short hairpin RNA (shRNA)-mediated removal of endogenous α-syn is toxic to SNpc neurons *in vivo*. Importantly, this toxicity is rescued by supplementation of α-syn. Thus, we posit that α-syn is not the primary toxic species in PD; rather, α-syn aggregation produces pathology by decreasing the pool of functional α-syn available to the cell resulting in a loss-of-function toxicity. To test this hypothesis we have developed a non-aggregatable isoform of α-syn (α-synC6). We predict that α-synC6 will rescue toxicity produced by α-syn loss-of-function (via knockdown or aggregation) by maintaining the pool of functional α-syn available to the cell. To first determine if α-synC6 retains function *in vivo*, we tested the ability of α-synC6 to rescue α-syn knockdown-induced toxicity as a surrogate of functionality. Rats received unilateral stereotaxic injections of recombinant adeno-associated virus (rAAV) expressing WT α-syn, α-synC6 or a GFP control into the SNpc. One month following transgene delivery, rats received unilateral injections of rAAV expressing shRNA directed toward α-syn or a control shRNA into the same SNpc. One month following α-syn shRNA administration, stereological cell counts in animals receiving rAAV-GFP or rAAV-WT α-syn showed a significant loss of tyrosine hydroxylase immunoreactive (THir) neurons in the injected SNpc. Animals that were injected with α-synC6 showed a partial rescue of THir neurons, as compared to GFP control animals. These results confirm that loss of functional α-syn is toxic to SNpc neurons and that α-synC6 retains functionality *in vivo* (as indicated by rescue from α-syn loss-of-function toxicity). Ongoing studies will test the ability of α-synC6 to rescue toxicity produced by WT α-syn overexpression, a condition that normally results in aggregation and subsequent loss of DA neurons within the SNpc. We hypothesize that this aggregation causes cytotoxicity by decreasing

availability of functional α-syn and that α-synC6 will abrogate this toxicity. Results from this and ongoing studies indicate that α-syn is necessary for the survival of DA neurons of the SNpc.

Disclosures: **M.J. Benskey:** None. **C. Jiang:** None. **C.E. Sortwell:** None. **N. Kanaan:** None. **F.P. Manfredsson:** None.

Nanosymposium

386. Neurodegeneration Mechanisms in Parkinson's Disease

Location: 147A

Time: Monday, November 17, 2014, 1:00 PM - 4:45 PM

Presentation Number: 386.04

Topic: C.03. Parkinson's Disease

Support: NIH Grant 1F31NSO84722-01A1

Title: Role of microRNA-155 in modulating inflammation in an alpha-synuclein model of Parkinson Disease

Authors: ***A. D. THOME**, A. S. HARMS, D. G. STANDAERT;
Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Parkinson disease (PD) is a neurodegenerative disorder characterized by loss of dopamine neurons in the substantia nigra pars compacta (SNpc) and aggregates of the protein alpha-synuclein (α-syn). Increasing evidence points to inflammation as a chief mediator of injury, however the role of α-syn in triggering and sustaining this inflammation remains unknown. Neuro-inflammatory responses have shown to be dynamically regulated by short, non-coding sequences called microRNAs (miRs). MiRs regulate messenger RNAs in a post-transcriptional fashion by binding to specific 3' untranslated regions resulting in repression or degradation of the transcript. We used an *in vivo* mouse model in which human α-syn is overexpressed by an AAV2 viral vector in mice (AAV2-SYN), resulting in elevated cytokine expression, reactive microgliosis, and progressive dopamine cell loss. At two weeks and four weeks after SNc AAV injection, we isolated miRNAs from the substantia nigra (injected and contralateral sides) and studied miRNA expression using a Qiagen PCR array containing probes for 84 microRNAs involved in either pro-inflammatory or anti-inflammatory regulation. We found that four of the microRNAs showed enhanced expression and two were reduced at two weeks post transduction. Among those which were increased was miR-155, which has been previously identified in other neurodegenerative diseases such as multiple sclerosis, amyotrophic lateral sclerosis, and Alzheimer disease. MiR-155 is known to target inflammatory pathways to

up-regulate IL-1, IL-6, and TNF-α and regulates anti-inflammatory pathways in reducing IL-10, Arg 1, IL-13R, and TGF-βR pathway proteins. We confirmed the increase in miR-155 using qPCR, which demonstrated a 1.55 ± 0.09 (p=0.01) fold increase of miRNA-155 at 2 weeks post transduction and a trend toward increase at 4 weeks (1.28 ± 0.28). We hypothesize that this enhanced miR-155 expression promotes and sustains the inflammatory environment associated with over-expression of human α-syn. If these data are supported by further in vivo experiments, microRNA-155 may be a target for novel therapeutic treatments for PD. Supported by APDA Advanced Center for PD Research at UAB and the Parkinson Association of Alabama

Disclosures: **A.D. Thome:** None. **A.S. Harms:** None. **D.G. Standaert:** None.

Nanosymposium

386. Neurodegeneration Mechanisms in Parkinson's Disease

Location: 147A

Time: Monday, November 17, 2014, 1:00 PM - 4:45 PM

Presentation Number: 386.05

Topic: C.03. Parkinson’s Disease

Support: Fondazioen grigioni per il Morbo di Parkinson, Milano, Italy

Title: Looking at microtubule dysfunction in Parkinson’s disease: From Parkin knockout mice to human iPSC-derived neurons

Authors: ***G. CAPPELLETTI**[1], D. CARTELLI[1], F. CASAGRANDE[1], C. DE GREGORIO[1], A. CALOGERO[1], J. SASSONE[2,3], H. OKANO[3], N. KUZUMAKI[3], A. AMADEO[1,2];
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Abstract: Parkinson’s disease is the major motor neurodegenerative disorder affecting 2-5% of population over sixties, worldwide. It is considered a multifactorial pathology and, among the various hypotheses about the molecular pathogenesis, a growing attention has been dedicated to the microtubule system. Indeed, the concept that microtubule dysfunctions can participate in, and perhaps lead to, Parkinson’s disease progression has been evoked by studies on toxin-based and genetic experimental models of the pathology. In the context of studies on genetic models of parkinsonism, here we investigated microtubule system in *Parkin* knockout mice and found that *Parkin* deficiency impacts microtubule dynamics *in vivo*. In knockout mice at different ages, we analyzed tubulin post-translational modifications that are usually used as marker of microtubule with different stability, being tyrosinated the most dynamic pool and detyrosinated or acetylated

more stable subsets. We showed that alterations accumulate over time with microtubule dysfunction preceding defects in axonal transport. Although the analysis of microtubule system in human sample has been substantially neglected, we recently reported that Parkinson's disease patient-derived fibroblasts, including fibroblasts from patients carrying *Parkin* (PARK2) mutations, have a reduced microtubule mass; noteworthy, both pharmacological treatment with microtubule-targeted drugs or genetic approaches restore the proper microtubule stability. In order to extend our previous observations, experiments are in progress to look at iPSC-derived dopaminergic neurons obtained from familial Parkinson's disease patients bearing *Parkin* deficiencies. Notably, acetylated microtubules appear fragmented inside neuronal process of patient-derived dopaminergic neurons, as compared to those of controls, in agreement with the microtubule destabilization observed in patient fibroblasts. Collectively, our data support the idea that *Parkin* deficiency significantly impacts microtubule stability, in mouse brain and in human neurons, and reinforce the hypothesis that microtubules can be a reliable culprit in causing Parkinson's disease.

Disclosures: G. Cappelletti: None. **D. Cartelli:** None. **F. Casagrande:** None. **C. De Gregorio:** None. **A. Calogero:** None. **J. Sassone:** None. **H. Okano:** None. **N. Kuzumaki:** None. **A. Amadeo:** None.

Nanosymposium

386. Neurodegeneration Mechanisms in Parkinson's Disease

Location: 147A

Time: Monday, November 17, 2014, 1:00 PM - 4:45 PM

Presentation Number: 386.06

Topic: C.03. Parkinson's Disease

Support: National Science Foundation of China (NSFC, 81371027)

National Science Foundation of China (NSFC, 81371027)

Title: Reductions of the activity of store-operated calcium channel by proteasome inhibition through autophagy induction

Authors: *S. WU[1], X.-L. KUANG[2], F. LIU[2], Y. LIU[1], H. CHEN[1];
[1]Sch. of Optometry and Ophthalmology, Wenzhou Med. Univ., Zhejiang, China; [2]Sch. of Optometry and Ophthalmology, Wenzhou Med. Univ., Wenzhou, China

Abstract: Amount of evidence suggest that proteasome inhibition plays roles in the neuronal degeneration in neurodegenerative disorders. Our previous studies indicated that sub-lethal doses of proteasome inhibitors induce dyshomeostasis of cytosolic and endoplasmic reticulum (ER) calcium level (Wu S, et al., J. Neurochemistry, 2009) and negatively impact the calreticulin/calnexin ER quality-control system in primary neuronal cultures (Kuang XL, et al., J. Neuroscience Research,2014). Here, we report that in primary rat neuronal cultures, proteasome inhibitors, including MG-132 (MG) and clasto-lactacystine-β-lactone (LA) at sub-lethal doses, reduced the protein level of stromal interaction molecule 1(STIM1), a component of SOCC channel; the effect may account for the reduced activity of store-operative calcium channel (SOCC) from the treatments, which were detected with ratiometric calcium imaging. Co-addition of Bafilomycin A1 (Baf),a specific inhibitor of vacuolar-type H^+-ATPase blocking the fusion between autophagosome and lysosome, reversed STIM1 protein level reduced by MG or LA. We further indicated that MG or LA activated autophagy in the primary rat neuronal cultures. Overall, our results suggest that proteasome inhibition may interfere with calcium dyshomeostasis via modulating SOCC channel, which may contribute to the reduction of cytosolic calcium or ER calcium observed in our early studies. Altogether, the series of studies suggest that elevation rather than reduction of cellular calcium is possibly a better strategy to prevent neuronal death at the early stage of neuronal injuries. The study was supported by National Science Foundation of China (NSFC,81371027), and by Chinese Ministry of Education (20133321120002). Correspondence should be directed to Dr. Shengzhou Wu, Ph.D, MD at wszlab@mail.eye.ac.cn

Disclosures: **S. Wu:** None. **X. Kuang:** None. **F. Liu:** None. **Y. Liu:** None. **H. Chen:** None.

Nanosymposium

386. Neurodegeneration Mechanisms in Parkinson's Disease

Location: 147A

Time: Monday, November 17, 2014, 1:00 PM - 4:45 PM

Presentation Number: 386.07

Topic: C.03. Parkinson’s Disease

Title: Glucocerebrosidase regulates motor and cognitive activities in mouse models of synucleinopathies

Authors: ***S. SARDI**, C. VIEL, J. CLARKE, M. CHAN, N. PANARELLO, C. TRELEAVEN, J. BU, L. STANEK, L. SWEET, M. PASSINI, J. DODGE, S. CHENG, L. SHIHABUDDIN; Genzyme, a Sanofi Co., Framingham, MA

Abstract: Mutations in *GBA1*, the gene encoding glucocerebrosidase (GC), represent a risk factor for developing synucleinopathies including Parkinson's disease (PD) and dementia with Lewy bodies (DLB). A partial loss-of-function hypothesis has been proposed to explain the role of *GBA1* mutations in the development of synucleinopathies. In line with this postulate, we had previously reported that augmentation of GC activity in the brain was efficacious in rescuing some of the disease manifestations of a mouse model of Gaucher-related synucleinopathy (Sardi et al., *PNAS* 108:12101, 2011) and at reducing alpha-synuclein levels in A53T-alpha-synuclein mice (Sardi et al. *PNAS* 110:3537, 2013). In addition, brains of PD patients with or without mutations in *GBA1* have been reported to present with reduced GC activity further suggesting a potential role of the enzyme activity in the pathogenesis of the disease. The present studies investigate whether a reduction in GC activity could lead to increased synuclein pathology *in vivo*. Two-month old mice were treated with a GC inhibitor, conduritol-B-epoxide (100mg/kg, 3 times/week, for 8 weeks). Treatment of A53T-alpha-synuclein mice with the GC inhibitor resulted in worsening of fine motor skills and cognitive function as determined by the nesting activity assay, pole test and conditional fear test. Lower GC activity also correlated with increased cortical alpha-synuclein levels as measured by ELISA (control: 3.8 ± 0.2 ng/mg prot, n=9; CBE-treated: 5.6 ± 0.4 ng/mg prot, n=9, $p<0.05$). In another set of experiments, an artificial miRNA was delivered into the brain of the Gaucher-related synucleinopathy mouse model ($Gba1^{D409V/D409V}$) to lower GC levels and the effects of further reducing the enzyme activity then measured. Animals developed normally until 15 weeks of age, when treated animals developed an overt aberrant motor phenotype. These included hyperactivity, ataxia, rotarod deficits, and abnormal stereotypic movements. These effects were rescued by the introduction of a miRNA-resistant GC, showing specificity for the GC knockdown strategy. In summary, these findings support the notion that reducing GC activity can contribute to the PD-like disease phenotype and further support our initial findings that increasing GC activity may represent a therapeutic strategy for the synucleinopathies.

Disclosures: **S. Sardi:** A. Employment/Salary (full or part-time):; Genzyme, a Sanofi Company. **C. Viel:** A. Employment/Salary (full or part-time):; Genzyme, a Sanofi Company. **J. Clarke:** A. Employment/Salary (full or part-time):; Genzyme, a Sanofi Company. **M. Chan:** A. Employment/Salary (full or part-time):; Genzyme, a Sanofi Company. **N. Panarello:** A. Employment/Salary (full or part-time):; Genzyme, a Sanofi Company. **C. Treleaven:** A. Employment/Salary (full or part-time):; Genzyme, a Sanofi Company. **J. Bu:** A. Employment/Salary (full or part-time):; Genzyme, a Sanofi Company. **L. Sweet:** A. Employment/Salary (full or part-time):; Genzyme, a Sanofi Company. **M. Passini:** A. Employment/Salary (full or part-time):; Genzyme, a Sanofi Company. **J. Dodge:** A. Employment/Salary (full or part-time):; Genzyme, a Sanofi Company. **S. Cheng:** A. Employment/Salary (full or part-time):; Genzyme, a Sanofi Company. **L. Shihabuddin:** A. Employment/Salary (full or part-time):; Genzyme, a Sanofi Company. **L. Stanek:** A. Employment/Salary (full or part-time):; Genzyme, a Sanofi Company.

Nanosymposium

386. Neurodegeneration Mechanisms in Parkinson's Disease

Location: 147A

Time: Monday, November 17, 2014, 1:00 PM - 4:45 PM

Presentation Number: 386.08

Topic: C.03. Parkinson's Disease

Title: Intracellular bacteria in post mortem Parkinson's brains

Authors: J. R. LEHESTE[1], *J. CHROSTOWSKI[1], K. RIVERA[1], A. MICELI[1], C. HUSKO[1], G. TORRES[1], M. K. SELIG[2], H. BRUEGGEMANN[3];
[1]New York Inst. of Technol. Col. of Osteop, Old Westbury, NY; [2]Massachusetts Gen. Hosp., Boston, MA; [3]Aarhus Univ., Århus, Denmark

Abstract: The human body hosts a complex system of an estimated one trillion microbes which outnumber our own cells by a factor of ten or more. Host-specific variations of microbial communities have been linked to pathological states and are intensely researched. Parkinson's disease (PD) is the most common movement disorder worldwide with well-established pathology yet poorly defined etiology and pathogenesis. As a consequence, the underlying circumstances of disease onset and progression are obscure in the vast majority (> 90%) of all PD cases. Interestingly, initial signs of PD-related cell death are seen in the olfactory bulb and the *area postrema* in the brainstem. Both areas lack a functional blood-brain-barrier allowing pharmaceuticals, chemicals and potential pathogens to enter the central nervous system (CNS). Thereafter, cellular damage and inflammation gradually progress towards the *substantia nigra* of the midbrain and, lastly, the cerebral cortex. Consistent with our hypothesis, this pattern is reminiscent of an insidious bacterial infection, benign but opportunistic in nature, transitioning from the body's surface into the CNS parenchyma and its cells. To test this hypothesis, we screened *post mortem* brain sections of advanced-stage PD and matching control individuals (n=12 per group) obtained from the Brain and Body Donation Program (Mayo Clinic, AZ) for common commensal bacteria using standard fluorescence immunohistochemical techniques. In parallel, we infected human dopaminergic neuroblastoma cells (SH-SY5Y) with various strains of bacterial microbiota and analyzed the outcome with standard transmission electron microscopy (TEM). In about 50% of all *post mortem* PD brain sections tested we found evidence of Gram-positive bacteria which could be visualized with immunofluorescence microscopy. Those were enriched in the cytoplasm of a small number of neurons in affected PD brains and absent in control brain tissue. TEM analysis of our cell-based experiment demonstrated the ability of certain commensal bacteria to invade human neuroepithelial cells. These results are the first step toward a previously unknown pathogenic mechanism for PD involving intracellular

bacteria in the human brain. Whereas cause and effect have yet to be determined in an appropriate *in vivo* model, our results bring emphasis to the importance of the human microbiome regarding brain health.

Disclosures: J.R. Leheste: None. **J. Chrostowski:** None. **K. Rivera:** None. **A. Miceli:** None. **C. Husko:** None. **G. Torres:** None. **M.K. Selig:** None. **H. Brueggemann:** None.

Nanosymposium

386. Neurodegeneration Mechanisms in Parkinson's Disease

Location: 147A

Time: Monday, November 17, 2014, 1:00 PM - 4:45 PM

Presentation Number: 386.09

Topic: C.03. Parkinson's Disease

Support: Division of Intramural Research, NINDS

Title: Profound putamen catecholamine depletion in Gaucher/Parkinson disease: Role of decreased vesicular sequestration

Authors: *D. S. GOLDSTEIN[1], P. SULLIVAN[1,2], N. TAYEBI[2], E. AFLAKI[2], E. SIDRANSKY[2];
[1]Clin. Neurocardiology Section CNP/DIR/NINDS/NIH, NINDS NIH, Bethesda, MD; [2]Mol. Neurogenetics Section, NHGRI, Bethesda, MD

Abstract: Background: In sporadic Parkinson disease (PD), profound putamen dopamine (DA) depletion importantly reflects decreased vesicular storage in residual dopaminergic terminals. Mutation in the gene encoding glucocerebrosidase, which causes Gaucher disease, predisposes to development of PD. We tested whether post-mortem mortem brain samples from patients with Gaucher/PD show neurochemical evidence of decreased vesicular sequestration of cytoplasmic catecholamines. **Methods:** Putamen tissue was obtained from 4 Gaucher/PD patients; catechol contents were compared to those in 25 control subjects. The tissue DA:DOPA concentration ratio was used as an index of vesicular storage of DA for a given amount of cytoplasmic DA synthesis. Since norepinephrine (NE) is produced only in vesicles, concentrations of NE and its main neuronal metabolite 3,4-dihydroxyphenylglycol (DHPG) also depend on vesicular uptake of cytoplasmic DA. **Results:** Gaucher/PD patients had drastically decreased putamen DA and norepinephrine (NE) contents compared to control subjects (by 98% and 95%, $p<0.0001$ each). DA:DOPA ratios and DHPG concentrations were also markedly decreased (by 97% and 95%) in the Gaucher/PD group ($p<0.0001$, $p=0.0001$). **Conclusions:** Putamen catecholamine depletion in

Gaucher/PD is associated with markedly decreased vesicular storage in residual catecholaminergic terminals. Since a shift from vesicular uptake to enzymatic oxidative deamination of cytoplasmic catecholamines would be expected to increase the production of cytotoxic aldehydes and hydrogen peroxide for a given amount of cytoplasmic DA, the sequestration-deamination shift might contribute to the neuronal death process in Gaucher/PD.

Disclosures: D.S. Goldstein: None. **P. Sullivan:** None. **E. Sidransky:** None. **E. Aflaki:** None. **N. Tayebi:** None.

Nanosymposium

386. Neurodegeneration Mechanisms in Parkinson's Disease

Location: 147A

Time: Monday, November 17, 2014, 1:00 PM - 4:45 PM

Presentation Number: 386.10

Topic: C.03. Parkinson's Disease

Title: Dopamine, hypochlorite and Parkinson's disease

Authors: N. J. MEHTA, K. A. BENINGO, *D. NJUS;
Biol. Sci., Wayne State Univ., Detroit, MI

Abstract: Parkinson's disease is a movement disorder caused by the death of dopaminergic neurons in the substantia nigra, but what kills these neurons is unknown. Oxidative stress, mitochondrial dysfunction, dopamine oxidation and microglial inflammation have all been implicated as contributing factors, but a mechanism relating them has been elusive. We suggest that hypochlorite produced by myeloperoxidase may link these disparate factors and play a central role in the progression of Parkinson's disease. At micromolar concentrations, hypochlorite reacts with the dopamine oxidation products cysteinyl-dopamine and cysteinyl-DOPAC and converts them into compounds that redox cycle. Myeloperoxidase, in the presence of its substrates H_2O_2 and Cl-, produces sufficient hypochlorite to do this as well. Hypochlorite is unique in its ability to form redox cycling compounds; other oxidants such as hydrogen peroxide and Fe^{3+} are not effective. Redox cycling occurs when a compound is reduced either chemically by ascorbic acid or enzymatically by NADH in the presence of mitochondria. The reduced compound then reacts spontaneously with O_2 to generate superoxide and hydrogen peroxide (H_2O_2). Redox cycling may be observed by following O_2 consumption with an oxygen electrode or by assaying the H_2O_2 product. The product made by hypochlorite treatment of cysteinyl-dopamine is nearly as effective at redox cycling as the synthetic compounds 3-methyl-5-anilino quinone and 9,10-phenanthrenequinone. Like these compounds, it is also cytotoxic at

micromolar concentrations. Furthermore, these compounds generate superoxide in cells as indicated by mitosox fluorescence suggesting that toxicity may be a consequence of oxidative stress caused by reactive oxygen species. This raises the possibility that hypochlorite, produced by microglial myeloperoxidase, creates redox cycling compounds that lead to oxidative stress and the consequent death of dopaminergic neurons in Parkinson's disease.

Disclosures: **N.J. Mehta:** None. **K.A. Beningo:** None. **D. Njus:** None.

Nanosymposium

386. Neurodegeneration Mechanisms in Parkinson's Disease

Location: 147A

Time: Monday, November 17, 2014, 1:00 PM - 4:45 PM

Presentation Number: 386.11

Topic: C.03. Parkinson's Disease

Title: Synergistic toxicity of synuclein and DOPAL in neurodegeneration

Authors: N. PLOTEGHER[1], I. TESSARI[1], E. FERRARI[2], S. GIROTTO[3], M. DALLA SERRA[4], E. GREGGIO[1], M. BISAGLIA[1], L. CASELLA[2], *L. BUBACCO[1];
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Abstract: The dopamine metabolite 3,4-dihydroxyphenylacetaldehyde (DOPAL) is highly reactive aldehyde of great interest in neurodegeneration. DOPAL is typically processed by the enzyme aldehyde dehydrogenase that is able to detoxify neurons by conversion of the toxic DOPAL to 3,4-dihydroxyphenylacetic acid. The toxicity ascribed to DOPAL acquires particular interest for neurodegeneration in Parkinson's disease, being the dopaminergic neurons the primary target of the disorder. In this frame, it was reported that DOPAL is accumulated in neurons in parkinsonian brains where it can chemically modify alpha-synuclein (aS), a protein strongly associated to familial and sporadic forms of Parkinson's disease. aS is a natively unfolded protein prone to form aggregates which are also found in parkinsonian brains. The goal of our study is the characterization of the synergistic toxic effect exerted by DOPAL and alpha-synuclein in Parkinson's disease. To this aim, we used a broad range of biophysical and biochemical techniques to characterize the chemical modification of aS due to the reaction with DOPAL and the heterogeneous ensemble of resulting oligomeric aggregated species. aS is modified by DOPAL mainly at lysine residues as verified by mass spec and NMR. The aS modifications lead to the formation of oligomers that were then characterized by both transmission electron microscopy and dynamic light scattering. These DOPAL dependent aS

oligomers are able to permeabilize artificial membranes inducing ions leakage. The formation and the toxicity of these aS oligomers have been observed also in a cellular model, M17 neuroblastoma cells.

Disclosures: **N. Plotegher:** None. **I. Tessari:** None. **E. Ferrari:** None. **S. Girotto:** None. **M. Dalla Serra:** None. **E. Greggio:** None. **M. Bisaglia:** None. **L. Casella:** None. **L. Bubacco:** None.

Nanosymposium

386. Neurodegeneration Mechanisms in Parkinson's Disease

Location: 147A

Time: Monday, November 17, 2014, 1:00 PM - 4:45 PM

Presentation Number: 386.12

Topic: B.07. Synaptic Transmission

Support: CIHR MOP 119347

CERC

Title: Introduction to novel retromer function in neurones and implications for the pathophysiology of parkinsonism and Alzheimer’s disease

Authors: *M. J. FARRER;
Med. Genet., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Endosomal sorting governs the fate of many physiologically important proteins through regulated trafficking from endosomes to other specialized compartments; including the trans-golgi network, lysosomes and the plasma membrane. Recent discoveries show that cargo is moved from endosomes by the formation of actin patches and tubule extension regulated by retromer subunit interactions (VPS35, 26 & 29) with RME-8/DNAJC13 and WASH-complex proteins. In neurones, retromer-associated endosomes are distributed throughout dendrites where they supply neurotransmitters via membrane fusion events into dendritic shafts. Both knock-down and overexpression of retromer subunits alters surface delivery of AMPA-type glutamate receptors and synaptic transmission. Clinicogenetic studies have linked mutations in retromer (VPS35) subunits and RME-8/DNAJC13 to familial parkinsonism, and mutant-dependent effects upon both synaptic connectivity and receptor trafficking have been defined. Furthermore, retromer interactions govern the traffic and processing of proteins important to dementia states including progranulin and the amyloid precursor protein APP. Here, four talks will showcase our

developing understanding of the cell and neurobiology of retromer-mediated sorting and the implications for late-onset neurodegenerative diseases. An emerging synthesis suggests that dysfunctional WASH/Retromer/RME-8 interactions and the resultant perturbations to synaptic receptor traficking may be the pathophysiological insult underlying several forms of late-onset parkinsonism and Alzheimer's disease.

Disclosures: M.J. Farrer: None.

Nanosymposium

386. Neurodegeneration Mechanisms in Parkinson's Disease

Location: 147A

Time: Monday, November 17, 2014, 1:00 PM - 4:45 PM

Presentation Number: 386.13

Topic: B.07. Synaptic Transmission

Support: MRC G0701444

Title: The retromer complex and associated proteins: New insights into the pathology of Parkinson's disease

Authors: *M. SEAMAN;
Cambridge Inst. for Med. Res., Univ. of Cambridge, Cambridge, United Kingdom

Abstract: The retromer complex is a conserved multimeric protein complex that mediates endosomal protein sorting and controls the localisation of many physiologically important proteins. Sorting of membrane proteins by retromer is performed by the cargo-selective complex (CSC) - a trimer of the VPS35, VPS29 and VPS26 proteins. In addition to sorting membrane proteins, the retromer CSC also functions as a hub for recruiting additional machinery required for endosomal protein sorting, one of which is the WASH complex that regulates formation of F-actin on endosomes. The retromer-WASH complex interaction occurs through VPS35 binding to the FAM21 protein of the WASH complex. An inherited mutation in VPS35 causes Parkinson's disease but the pathophysiological mechanism of the PD-causing VPS35 mutation is unknown. I will present data showing that the PD-causing mutation in VPS35 impairs binding to the WASH complex leading to endosomal protein sorting defects. Additionally, I will also report the association of the FAM21 protein with RME-8, another recently identified PD-causing gene and show the effects of loss of RME-8 function on endosome morphology. Thus, retromer-mediated endosomal protein sorting, and the proteins that mediate this process, may constitute an attractive avenue for future studies of the pathology of PD.

Disclosures: M. Seaman: None.

Nanosymposium

386. Neurodegeneration Mechanisms in Parkinson's Disease

Location: 147A

Time: Monday, November 17, 2014, 1:00 PM - 4:45 PM

Presentation Number: 386.14

Topic: B.07. Synaptic Transmission

Support: Agreement #42800 (Anonymous)

Title: The retromer-associated endosome in the pathophysiology of Alzheimer's disease

Authors: *S. A. SMALL;
Taub Inst. Res. & Alzh Dis & Aging Brain, Columbia Univ. Med. Ctr., New York, NY

Abstract: The retromer was first implicated in Alzheimer's disease (AD) guided by the disease's regional vulnerability within the hippocampal circuit. Since then a range of studies in human genetics, animal models, and cell culture have established that defects in the retromer-associated endosome play a pathogenic role in AD-- upstream to amyloid, tangle, and glial pathologies. After a brief review of these older studies, 4 new lines of investigation will be discussed: A) Studies showing that defects in the retromer-associated endosome play a role in three hallmark features of AD. 1. The accumulation of neurotoxic fragments of the amyloid precursor protein (APP). 2. The neurotoxic processing of the tau protein. 3. Microglia defects. B) Studies identifying which specific elements of the retromer complex are most linked to APP processing in the brain. C) Studies that have isolated a novel group of compounds, 'retromer pharmacological chaperones', that correct defects in the retromer-associated endosome observed in AD and Parkinson's disease. D) Studies that suggest how the retromer-associated endosome can explain regional vulnerability in AD.

Disclosures: S.A. Small: None.

Nanosymposium

386. Neurodegeneration Mechanisms in Parkinson's Disease

Location: 147A

Time: Monday, November 17, 2014, 1:00 PM - 4:45 PM

Presentation Number: 386.15

Topic: B.07. Synaptic Transmission

Support: CIHR (MOP 119347; to MJF, AM)

Title: Parkinsonism, mutant VPS35, and novel retromer functions in neurons

Authors: *A. J. MILNERWOOD[1], L. N. MUNSIE[2], P. SEIBLER[3], D. BECCANO-KELLY[2], M. VOLTA[2], C. KLEIN[3], M. J. FARRER[2];
[1]Neurology, Ctr. for Applied Neurogenetics & Brain Res. Ctr., [2]Univ. of British Columbia, Vancouver, BC, Canada; [3]Univ. of Lubeck, Lubeck, Germany

Abstract: Vacuolar protein sorting 35 (VPS35) is a core component of the retromer complex, crucial to endosomal protein sorting and intracellular trafficking. Until recently, little was known about retromer function in neurons but we recently linked a VPS35 mutation (p.D620N) to familial parkinsonism. Here we show that VPS35 is involved in the trafficking of excitatory AMPA-type neurotransmitter receptors (AMPARs). We examined the affect of the p.D620N mutation on neuronal function. While some measures are unaffected by the p.D620N mutation, we found altered intracellular trafficking and subcellular localization of mutant VPS35, in addition to altered synaptic transmission, AMPAR surface expression, and AMPAR recycling. Perturbations to synaptic function in the presence of the p.D620N mutation may produce chronic pathophysiological stress and subsequent neurodegeneration. Furthermore mutations in other retromer interacting proteins, such as RME-8, are also linked to familial parkinsonism. The data suggest that retromer, and resultant synaptic, dysfunction may underlie several forms of parkinsonism.

Disclosures: A.J. Milnerwood: None. **L.N. Munsie:** None. **P. Seibler:** None. **D. Beccano-Kelly:** None. **M. Volta:** None. **C. Klein:** None. **M.J. Farrer:** None.

Nanosymposium

387. Cocaine: New Findings on Neural Mechanisms

Location: 140A

Time: Monday, November 17, 2014, 1:00 PM - 3:00 PM

Presentation Number: 387.01

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA

Title: CYFIP2 is a key regulator of cocaine response

Authors: *V. KUMAR[1], J. TAKAHASHI[2];
[1]Neurosci., Univ. of Texas, Southwestern Med. Ctr., Dallas, TX; [2]Neurosci., UT Southwestern, Dallas, TX

Abstract: Neuronal circuitry is a key regulator of behaviors including addiction. Acute and sensitized response to drugs of abuse and addiction is regulated by the mesolimbic reward circuit, consisting of ventral tegmental area in the midbrain, the nucleus accumbens in the striatum, and medial prefrontal cortex. Neuromodulation of this circuit can occur through structural and functional plasticity and is thought to be key to addiction. Correlative changes in neuronal structure have been described as a result of psychostimulant administration. Whether these structural changes are necessary for modulation of cocaine response and addiction has been controversial. Discovery of genes and pathways regulating structural plasticity in the reward circuitry can shed important insights to addiction. Here we use positional cloning and quantitative genetics to show that Cyfip2, a component of the WAVE regulatory complex, is mutated in a commonly used mouse strain, C57BL/6N. This mutation in CYFIP2 leads to diminished acute and sensitized cocaine response when compared to the reference C57BL/6J substrain. The WAVE regulatory complex is an actin nucleation factor which regulates neuronal structure. The C57BL/6N mutation in CYFIP2 greatly destabilizes the protein and leads to a decrease in dendritic spines in the nucleus accumbens. This decrease in excitatory post synaptic sites leads to decreased frequency, but not amplitude, of mEPSC in the nucleus accumbens. We will present novel data on the analysis of Cyfip2 and Cyfip1 conditional knockouts. Thus, using forward genetics, we identify a novel regulator of cocaine response in mammals. Our data links CYFIP2 and the WAVE regulatory complex to cocaine response and neuronal structure in the reward circuitry.

Disclosures: V. Kumar: None. **J. Takahashi:** None.

Nanosymposium

387. Cocaine: New Findings on Neural Mechanisms

Location: 140A

Time: Monday, November 17, 2014, 1:00 PM - 3:00 PM

Presentation Number: 387.02

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant DA003906

NIH grant DA012513

NIH grant DA015369

Title: Let it SNO: S-nitrosylation of matrix metalloproteinases following cocaine exposure mediates vulnerability to cocaine relapse

Authors: *A. W. SMITH[1], M. D. SCOFIELD[1], M. LORANG[2], P. W. KALIVAS[1]; [1]Neurosciences, Med. Univ. of South Carolina, Charleston, SC; [2]Col. of Charleston, Charleston, SC

Abstract: Chronic cocaine exposure produces neuroplasticity within the nucleus accumbens core (NAcore) that leads to increased vulnerability to relapse, even after protracted abstinence. Matrix metalloproteinases (MMPs) are pro-plasticity enzymes that degrade the extracellular matrix in order to promote synaptic growth and reorganization. Previous data from our lab show that both MMP-2 and MMP-9 are required for cue-induced reinstatement of cocaine seeking. Following extinction of cocaine self-administration there is a constitutive upregulation of MMP-2 in the NAcore, which in turn produces a persistent potentiation of synapses on medium spiny neurons (MSNs; as measured by dendritic spine head diameter and AMPA:NMDA ratio). Additionally, cocaine-conditioned cues produce a transient induction of MMP-9 activity, coinciding with a transient synaptic potentiation onto MSNs. However, it is unknown how either of these two enzymatic inductions occurs. MMPs are secreted in an inactive pro-form, in which a critical Zn^{2+} molecule is positioned between a single cysteine residue in the pro-domain, and 3 cysteine residues in the enzyme active site. The enzyme is activated when Zn2+ interaction with the pro-domain cysteine is disrupted, allowing Zn^{2+} to fully coordinate within the active site. One process by which this occurs is S-nitrosylation of the pro-domain cysteine by nitric oxide. We hypothesized that cocaine exposure induces neuronal nitric oxide synthase (nNOS) activity that in turn increases activity of both MMP-2 and MMP-9 through S-nitrosylation. In support of this hypothesis, we have shown that inhibition of nNOS reduces both constitutive and cue-induced inductions of MMP activity, measured by *in vivo* zymography. Furthermore, by immunoprecipitating each MMP and probing for S-NO-cysteine, we were able to verify increased S-nitrosylation of these enzymes following extinction and reinstatement. nNOS inhibition was also found to block cue-induced reinstatement. Taken together, these findings indicate that S-nitrosylation of metalloproteinases is a novel pathway mediating synaptic potentiation following repeated cocaine exposure. Interestingly, nNOS is only expressed in ~1% of cells in the striatum. Ongoing experiments will utilize NOS1-Cre transgenic mice to retrogradely label afferent connections onto nNOS+ neurons, and to target DREADDs to these neurons to test their ability to manipulate MMP activity.

Disclosures: A.W. Smith: None. **M.D. Scofield:** None. **M. Lorang:** None. **P.W. Kalivas:** None.

Nanosymposium

387. Cocaine: New Findings on Neural Mechanisms

Location: 140A

Time: Monday, November 17, 2014, 1:00 PM - 3:00 PM

Presentation Number: 387.03

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH R00 DA026994

Research and Education Initiative Fund, a component of the Advancing a Healthier Wisconsin Endowment at the MedicalCollege of Wisconsin

Title: Within animal comparison of neuronal activation patterns associated with novelty and cocaine in thenucleus accumbens and prefrontal cortex

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Abstract: Novelty seeking is a personality trait associated with an increased vulnerability for substance abuse. In rodents, 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 have actions within the prefrontal cortex (PFC) and nucleus accumbens (NAc), little is known as to whether the same neural ensembles are engaged by these two stimuli. In this project, we wanted to determine if the same neurons were activated during both novelty and cocaine administration. Using the TetTag mouse model, we labeled active neurons at two different experimental time points within the same animal. The TetTag mouse model is a dual transgenic reporter line that allows for long lasting temporally controlled tagging of active neurons. All TetTag animals were maintained on chow containing doxycycline (dox, to inhibit tagging) prior to the start of the experiment. When dox was removed, experimental animals were exposed to novel stimuli, followed by a single cocaine injection in the home cage three days after resumption of dox. The experimental animals were perfused and tissue was taken two hours after the injection of cocaine. The control mice had the same dox regimen and perfusion schedule but remained in the home cage for the duration of the experiment. To visualize the cocaine-activated neurons, we performed immunohistochemistry for Fos and utilized the TetTag reporter to detect novelty-activated neurons. Using the TetTag LacZ model, which expresses long lasting beta-galactosidase (beta-gal) in active neurons, we found there were more beta-gal positive neurons in both the infralimbic (IL) and prelimbic (PrL) subregions of the PFC as well as in the core and shell of the NAc in experimental animals compared to controls. Additionally, in the PrL PFC, NAc core, and NAc shell we found significantly greater co-expression of beta-gal and Fos in

experimental mice relative to controls. Subsequent analysis of observed co-expression relative to that observed by chance indicated that this dual labeling was significantly greater than chance in the NAc core and shell. The data to date suggest that novelty and cocaine activate a similar network of neurons in the NAc, but are inconclusive as to whether or not this occurs in the PFC. Ongoing experiments are investigating if similar trends are observed when novelty and drug rewards are self-administered.

Disclosures: **N. Nawarawong:** None. **M.J. Muelbl:** None. **Y. Lim:** None. **C.M. Olsen:** None.

Nanosymposium

387. Cocaine: New Findings on Neural Mechanisms

Location: 140A

Time: Monday, November 17, 2014, 1:00 PM - 3:00 PM

Presentation Number: 387.04

Topic: C.17. Drugs of Abuse and Addiction

Support: T32-AA007583-11

Title: Activin/Smad3 induction in the nucleus accumbens mediates cocaine relapse

Authors: ***A. M. GANCARZ**[1], G. SCHROEDER[1], D. N. ADANK[1], C. PANGANIBAN[1], M. S. HUMBY[1], K. BRAUNSCHEIDEL[1], D. THORN[1], A. J. ROBISON[2], J.-X. LI[1], R. L. NEVE[3], D. M. DIETZ[1];
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Abstract: The addicted phenotype is characterized by a long-lasting, chronic, relapsing disorder that persists despite long periods of abstinence, suggesting that the underlying molecular changes are stable and enduring. Many of the long-term effects of cocaine have been shown to be dependent on alterations in gene expression that lead to prolonged adaptations, such as structural changes of medium spiny neurons in the reward circuitry of the brain. We have previously shown that withdrawal from cocaine self-administration (but not non-contingent exposure to cocaine) activates TGF-Beta superfamily signaling in the nucleus accumbens (NAc). Here, we investigate Activin receptor-mediated signaling via downstream Smad3 protein following withdrawal from cocaine self-administration. The Activin type II receptor was increased in the NAc at both the mRNA and protein levels following 7 days of withdrawal from cocaine self-administration. Direct pharmacologic antagonism of the Activin receptor in the NAc resulted in

decreased self-administration and attenuated drug-induced reinstatement/relapse behaviors without affecting locomotor activity or food-maintained responding. Pharmacologic activation of the Activin receptor via microinjections of Activin A into the NAc potentiated cocaine-primed reinstatement, without affecting locomotor activity. Withdrawal from cocaine self-administration also increased the expression of phosphorylated-Smad3, the downstream intracellular mediator of Activin signaling. Using viral-mediated gene transfer, we found that overexpression of Smad3 in the NAc potentiated cocaine-primed reinstatement. Importantly, blockade of Smad3 signaling via overexpression of a dominant negative Smad3 (dn-Smad3) attenuated cocaine self-administration. Taken together, these data indicate that Activin/Smad3 signaling is induced following withdrawal from cocaine self-administration and such regulation may be a key molecular mechanism underlying behavioral plasticity. Our ongoing studies are focused on examining how these molecular pathways regulate downstream transcriptional events and structural plasticity following withdrawal from self-administration.

Disclosures: **A.M. Gancarz:** None. **G. Schroeder:** None. **D.N. Adank:** None. **C. Panganiban:** None. **M.S. Humby:** None. **D. Thorn:** None. **A.J. Robison:** None. **J. Li:** None. **R.L. Neve:** None. **D.M. Dietz:** None. **K. Braunscheidel:** None.

Nanosymposium

387. Cocaine: New Findings on Neural Mechanisms

Location: 140A

Time: Monday, November 17, 2014, 1:00 PM - 3:00 PM

Presentation Number: 387.05

Topic: C.17. Drugs of Abuse and Addiction

Support: DA-031734

Title: Social stress, impulsivity and escalated cocaine taking: A social model of inhibitory control toward threatening stimuli in rats

Authors: *C. O. BOYSON, A. R. BURKE, K. A. MICZEK;
Psychology, Tufts Univ., Medford, MA

Abstract: Many procedures encompass features of decision-making, attention, and inhibitory control when modeling impulsivity. The new proposed model incorporates all three features while shifting the context from a traditional operant conditioning setup to a threatening social interaction. We focus on the latency to seek social contact, the duration of aggressive interactions, and the latency to terminate and escape these interactions. Male Long-Evans rats

were exposed to nine intermittent social defeat episodes over 21 days in an apparatus with three compartments permitting entry and escape. We categorized individual subjects based on a temporal characteristic of behavioral inhibition (slow versus fast) associated with the latency to enter a threat zone near an aggressor. Upon characterization we assessed saccharin preference, novelty-induced locomotion, cocaine-induced locomotion and intravenous cocaine taking. In a separate group of rats we measured total brain-derived neurotrophic factor (BDNF) and Trk-B receptor mRNA via qRT-PCR in tissue from the hippocampus and prefrontal cortex one hour after the last defeat. In a third group of rats we assessed impulsive behaviors following social stress under the Go/No-Go task. Those individuals consistently falling in the lower third of the ranks showed reduced inhibitory control characterized by fast entry times when entering the threat zone. Importantly, reduced inhibitory control during a threatening encounter was a highly predictive phenotype to engage in escalated cocaine taking during a 24-hour binge. Conversely, individuals consistently falling in the upper third of the ranks showed a greater inhibition during threatening stimuli. These rats also showed a significantly blunted total BDNF and Trk-B receptor mRNA in the hippocampus, suggesting differences in synaptic plasticity within the hippocampus between slow or fast groups. Lastly, social defeat stress increased impulsive-like decision making during the Go/No-Go task as assessed by premature responses, by "hits", and by false alarms for saccharin rewards. These results suggest that social defeat stress can promote impulsive-like decisions which may be associated with vulnerability to escalated cocaine taking during binge conditions.

Disclosures: C.O. Boyson: None. **A.R. Burke:** None. **K.A. Miczek:** None.

Nanosymposium

387. Cocaine: New Findings on Neural Mechanisms

Location: 140A

Time: Monday, November 17, 2014, 1:00 PM - 3:00 PM

Presentation Number: 387.06

Topic: C.17. Drugs of Abuse and Addiction

Support: Italian Ministry of Health, Young Investigator 2009

Title: Exposure to social adverse environment in early age induces vulnerability to drug-addiction in adulthood

Authors: *V. CAROLA[1], L. LO IACONO[2], A. VALZANIA[1], F. VISCO-COMANDINI[3], L. ROSCINI[3], A. FELSANI[4], E. ARICÒ[5], S. CABIB[3], S. PUGLISI-ALLEGRA[3];

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Abstract: Drug addiction is a chronic relapsing pathology that emerges only in a small proportion of drug users. A great deal of interest has been placed on the quality of the environment experienced during early age as a modulator of the susceptibility to drug-addiction in adulthood (Sinha, R., Ann NY Acad Sci, 2008;1141:105-130). In this context, it has been shown that the exposure to childhood physical maltreatment correlates with the abuse of a number of substances, including alcohol, cocaine, heroin, and marijuana in adulthood (Khoury et al., Depression and Anxiety, 2010; 27:1077-86). Whilst several animal studies showed a similar modulation of acute and/or chronic stress on the escalation of drug abuse, the long term effects of an early “social aversive” environment in inducing vulnerability to addiction and relapse to drug seeking after withdrawal has never been modeled in rodents. In our laboratory, we exposed mouse pups to an adult aversive male mouse (potential aggressive) in its resident cage for 30' per day from postnatal day 14 to 22. When adult stressed and control mice were then tested in the Conditioned Place Preference (CPP) paradigm, we observed an increased cocaine-induced CPP and reinstatement in stressed mice. To identify the molecular substrates underlying this behavioural phenotype, genome-wide RNA expression analyses (RNA-Sequencing and Genome-Wide Microarray) were performed in both brain and blood of stressed and control mice sacrificed at CPP reinstatement. These analyses showed that an high number of transcripts were differentially expressed between stressed and control mice in brain and blood. Future experiments aimed to clarify how these transcript differences contribute to the observed behavioral phenotype in CPP, will provide relevant and important information on the biological modifications involved in the susceptibility to substance use disorders associated with an adverse early environment.

Disclosures: V. Carola: None. **L. Lo Iacono:** None. **A. Valzania:** None. **F. Visco-Comandini:** None. **L. Roscini:** None. **A. Felsani:** None. **E. Aricò:** None. **S. Cabib:** None. **S. Puglisi-Allegra:** None.

Nanosymposium

387. Cocaine: New Findings on Neural Mechanisms

Location: 140A

Time: Monday, November 17, 2014, 1:00 PM - 3:00 PM

Presentation Number: 387.07

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA025785 (SW)

Title: A 5-HT1A receptor-mediated mechanism in the bed nucleus of the stria terminalis is associated with cocaine-seeking behavior

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Abstract: Experiences of stressful or anxiogenic situations are significant contributing factors underlying chronic relapse to drug use in addiction. Stress responses are known to involve the bed nucleus of the stria terminalis (BNST), a brain area richly innervated with serotonin. Serotonin (5-HT) plays a key role in mood and anxiety. As such, the etiology of addictive behaviors may be associated with an altered 5-HT system, particularly in the BNST. The present study, therefore, tested the hypothesis that increased 5-HT signaling in the BNST, via activation of 5-HT1A receptors with 8-hydroxy-N,N-dipropyl-2-aminotetralin (8-OH-DPAT), would decrease the display of addictive behaviors. In rodents, extended daily access to cocaine causes robust escalation of self-administration behavior, providing a valid model of human compulsive cocaine use in addiction. The present study investigated the effect of intra-BNST infusion of 8-OH-DPAT on cocaine self-administration in this rodent model using male Wistar rats. In addition, we assessed the effect of intra-BNST 8-OH-DPAT administration on stress-induced reinstatement of cocaine seeking in the conditioned-place preference paradigm, using the pharmacological stressor, yohimbine. Lastly, we examined the role of the BNST 5-HT system in mediating cocaine-induced hyperlocomotor activity. Selective activation of 5-HT1A receptors in the BNST dose-dependently attenuated the heightened motivation to take cocaine in rats with extended access, but not in control rats with limited daily access to cocaine. Concurrently, stress-induced reinstatement of cocaine seeking was inhibited by intra-BNST 8-OH-DPAT pretreatment. Further still, intra-BNST 8-OH-DPAT infusion significantly and selectively decreased cocaine-induced hyperactivity, without compromising general open-field locomotor behavior. In summary, the results suggest that a 5-HT1A receptor-mediated mechanism in the BNST underlies escalated motivation for cocaine and cocaine seeking in rodent models of drug abuse. Moreover, we found first evidence highlighting a role of the BNST 5-HT system in regulating the psychostimulant effect of cocaine, which may indicate its role in the hypothesized incentive sensitization process to cocaine in addiction. Collectively, these data propose BNST 5-HT1A receptors are a novel target for pharmacological intervention of cocaine abuse.

Disclosures: S.R. Wright: None. **I. You:** None. **S. Wee:** None.

Nanosymposium

387. Cocaine: New Findings on Neural Mechanisms

Location: 140A

Time: Monday, November 17, 2014, 1:00 PM - 3:00 PM

Presentation Number: 387.08

Topic: C.17. Drugs of Abuse and Addiction

Support: Medical Research Council G1000008

Title: GABRA2 variations influence subjective responses to methylphenidate and methylphenidate-facilitation of conditioned reinforcement

Authors: *D. N. STEPHENS, C. I. DIXON, L. TRICK, H. S. CROMBAG, S. L. KING, T. DUKA;
Univ. of Sussex, Brighton, United Kingdom

Abstract: Variations in the GABRA2 gene, encoding α2 subunits of GABAA receptors, have been associated with risk for cocaine addiction, but the mechanisms by which variations in non-coding regions of GABRA2 translate into risk for addictions are not understood. Mice with deletion of gabra2 show deficits in the ability of psychostimulants to facilitate instrumental responding for discrete tone and light cues previously conditioned to food rewards (conditioned reinforcement; CRf), offering a potential link. We established two novel methods to study CRf in healthy human volunteers, characterised as carrying cocaine addiction "risk" and "protective" SNPs within GABRA2, and studied methylphenidate's ability to facilitate CRf in both tasks, which were either independent of, or dependent on rate of responding. In parallel, we assessed the volunteers for their subjective responses to methylphenidate (20 mg). In order to provide a link to gabra2 -/- mice, we also studied the ability of methylphenidate (0.3 - 3 mg/kg, i.p.) to facilitate responding for CRf in wildtype and α2 knockout mice. As previously shown with cocaine, methylphenidate increased responding for CRf in wildtype, but not α2 KO mice. Individuals carrying "protective" GABRA2 SNPs felt stimulated and restless following methylphenidate, while individuals carrying "risk" SNPs did not, suggesting a relative lack of a subjective stimulant response in individuals carrying the "risk" SNPs. In keeping, in "protective", but not in "risk"-SNP carriers, methylphenidate potentiated the number of viewings of the CS+ (conditioned reinforcer) in the CRf task version requiring repeated responding. In the rate-independent measure, methylphenidate failed to increase time spent viewing the CS+ in either group. Thus, "risk" SNP carriers were insensitive to methylphenidate's effects both on mood or in facilitating CRf. Mice with deletion of the gene were also insensitive to methylphenidate's effects on responding for conditioned reinforcement. The parallel observations of loss of methylphenidate's effects in KO mice and "risk" SNP humans, suggests that SNPs associated with the "risk" for cocaine addiction may functionally impair GABAergic transmission in circuits employing α2 GABAA receptors. Circuits employing GABAA α2 subunits may thus protect against risk for addictions.

Disclosures: **D.N. Stephens:** None. **C.I. Dixon:** None. **L. Trick:** None. **H.S. Crombag:** None. **S.L. King:** None. **T. Duka:** None.

Nanosymposium

390. Pain Imaging: From Neural Circuits to Perception

Location: 147B

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 390.01

Topic: D.08. Pain

Support: Harvard Catalyst Advanced Imaging Pilot Grant

1UL1TR001102-01

8UL1TR000170-05

1UL1RR025758-04

Title: Evidence of brain glial activation in chronic low back pain patients

Authors: ***M. L. LOGGIA**[1], D. CHONDE[1], O. AKEJU[1], G. ARABASZ[1], C. CATANA[1], R. EDWARDS[2], E. HILL[3], S. HSU[1], D. IZQUIERDO-GARCIA[1], R.-R. JI[4], V. NAPADOW[1,5], M. RILEY[1], A. WASAN[6], N. ZÜRCHER[1], B. ROSEN[1,5], J. HOOKER[1];
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Abstract: A plethora of animal studies clearly supports a central role for glial cells in the establishment and/or maintenance of persistent pain (Ji RR et al., Pain 2013). However, the role of glial activation in human pain disorders remains unknown. In this study, 9 patients diagnosed with cLBP and 9 healthy controls had their brain scanned with [^{11}C]PBR28, a novel PET radioligand that activated microglia and astrocytes. The scanned participants were identified from a larger pool of subjects (n=44) so that each patient was matched to a healthy control for the Ala147Thr polymorphism in the TSPO gene (7 Ala/Ala, 2 Ala/Thr in both groups), sex (5 M, 4 F in both groups) and age (mean ± SD: cLBP = 48.8 ± 12.3, controls = 49.9 ± 12.7; p=0.37). This matched-pair design was adopted as TSPO polymorphism strongly predicts binding affinity for [^{11}C]PBR28 (Owen DR et al., J cereb blood flow metab 2012), while the effects of sex and age on ligand binding are unknown. Brain imaging was performed on a Siemens 3T integrated

PET/MR scanner, which allowed the acquisition of high-resolution T1-weighted anatomical volumes, simultaneously to PET data. Standardized Uptake Values, normalized to whole brain (SUVR), were computed from 60-90min post-injection. A voxelwise, whole-brain, matched-pair analysis was conducted to compare non-linearly spatially normalized SUVR maps across groups, using permutation testing (randomise, 10,000 permutations), and threshold-free cluster enhancement (corrected threshold of p=0.05). [^{11}C]PBR28 SUVRs were significantly higher in patients than controls in thalamus, the putative somatosensory representations of the lumbar spine (in the central sulcus) and leg (in the paracentral lobule), and in the precentral gyrus. This activation pattern is consistent with the majority of patients suffering from pain in the lower back and leg(s), and is suggestive of somatotopically organized glial activation in the primary somatosensory and motor cortices. Controls did not demonstrate higher ligand binding in any region compared to patients. This study is the first to demonstrate the occurrence of glial activation in the brain of chronic pain patients. Given the putative role of activated glia in many challenging issues associated with pain management, such as the induction of opioid-induced hyperalgesia and tolerance, the present findings offer far-reaching clinical implications that may serve to guide future studies of the pathophysiology and management of a variety of persistent pain conditions.

Disclosures: **M.L. Loggia:** None. **D. Chonde:** None. **O. Akeju:** None. **G. Arabasz:** None. **C. Catana:** None. **R. Edwards:** None. **E. Hill:** None. **S. Hsu:** None. **D. Izquierdo-Garcia:** None. **R. Ji:** None. **V. Napadow:** None. **M. Riley:** None. **A. Wasan:** None. **N. Zürcher:** None. **B. Rosen:** None. **J. Hooker:** None.

Nanosymposium

390. Pain Imaging: From Neural Circuits to Perception

Location: 147B

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 390.02

Topic: D.08. Pain

Support: Department of Neural and Pain Sciences, University of Maryland School of Dentistry

Title: The frontal polar cortex may encode the cognitive load of pain

Authors: M. MOAYEDI[1], T. MEEKER[2], S. KHAN[2], *D. SEMINOWICZ[2];
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[2]Dept of Neural & Pain Sci., Univ. of Maryland, Baltimore, Baltimore, MD

Abstract: The interaction of pain and cognitive performance has been demonstrated in healthy subjects as a reduction in task performance (i.e., longer reaction times) when concurrently presented with a noxious stimulus. Pain can interrupt or detract from the ability to perform a cognitive task, possibly because it is difficult to disengage from the painful stimulus. Difficult tasks that presumably require more cognitive resources could briefly reduce subjective pain ratings. Therefore, pain can affect cognitive performance, and cognitive performance can modulate pain ratings. These processes have been summarized in the Neurocognitive Model of Attention to Pain, which describes a "bottom-up capture of attention by pain", whereby pain grabs one's attention to signal a relevant and threatening stimulus. However, it remains unclear whether pain actually has a cognitive load, or whether pain simply modulates task performance through different mechanisms. Recent studies have implicated a role for the medial frontal polar cortex (mFPC) in complex executive cognitive functions including cognitive branching, the ability to put a pending task on hold to execute an ongoing one. Task priority is based on the perceived salience of the competing tasks. Therefore, we investigated whether pain has a cognitive load by determining whether it engages the cognitive branching system. 14 healthy subjects(7f, 8m) underwent structural and functional MRI scanning, quantitative sensory testing and completed questionnaires over two different testing sessions, at least two weeks apart. We induced pain with a 10% capsaicin cream while applying a warm thermode at 39 to 42 °C, adapted to each subject to produce a mild to moderate pain (Bencherif et al 2002 Pain). Subjects performed the Multi Source Interference Task (MSIT) while receiving no stimulus (scan 1) or during continuous pain (scan 2). fMRI scans were 5min 25s (TR of 2.5s, resolution 1.8x1.8x4 mm, 3T Siemens Tim-Trio). We performed a correlation analysis between mFPC activity and pain ratings during the task. Additionally, we compared mFPC activity between the two sessions with a paired t-test (pain-no pain). Average pain ratings in scan 2 were 33.3 ± 4.2 on a 0 to 100 scale. We found that 8/14 subjects' pain interfered task performance (P-type behavior; Seminowicz et al 2004 Pain). Additionally, we found that mFPC activity was correlated with pain intensity, and that, in the same region, there were BOLD differences in the mFPC between the pain and no pain MSIT sessions. These findings are in line with the concept of cognitive branching, that the cognitive load of pain interacts with and modulates task performance.

Disclosures: **M. Moayedi:** None. **S. Khan:** None. **T. Meeker:** None. **D. Seminowicz:** None.

Nanosymposium

390. Pain Imaging: From Neural Circuits to Perception

Location: 147B

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 390.03

Topic: D.08. Pain

Support: CIHR Fellowship Award

Royal Society University Research Fellowship: UF061613

Title: Dishabituation of nociceptive ERPs is dependent on changes of stimulus location in egocentric coordinates

Authors: *M. MOAYEDI[1], G. DI STEFANO[2], G. IANNETTI[1];
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Abstract: Introduction. Event-related potentials (ERPs) elicited by transient nociceptive stimuli in humans are largely sensitive to bottom-up novelty induced, for example, by changes in stimulus attributes (e.g., modality or spatial location) within a stream of repeated stimuli. Here we tested (1) whether a heterosegmental change in stimulus location elicits an ERP dishabituation, and, if so, (2) whether the dishabituation is dependent on the distance between the stimulated body territory in egocentric coordinates. **Methods.** Seven healthy right-handed volunteers took part in the study. EEG activity was recorded with 32 Ag-AgCl electrodes during four different blocks of laser stimulation. In each block, 30 trains of laser stimuli were presented. Each train consisted of three stimuli (S1–S2–S3, a triplet) delivered to either the foot dorsum (F) of the hand dorsum (H), at a constant inter-stimulus interval of 1 s. There were two stimulation patterns: Hand-Hand-Hand (HHH, no change), and Foot-Foot-Hand (FFH, change). In two of the four blocks, participants received HHH and FFH triplets while sitting with the hand resting on a table and the foot on the floor, resulting in an approximate distance of 1 m between the stimulated hand and foot (‘far’ condition). In the other two blocks, participants received HHH and FFH triplets while sitting, but with the hand and the foot placed close to each other on a platform, resulting in an approximate distance of 10 cm between the stimulated hand and the foot (‘near’ condition). Amplitudes of the N2 and P2 of the S3-LEP were analysed using a two-way ANOVA, with ‘change’ (two levels: yes, no) and ‘posture’ (two levels: near, far) as experimental factors. **Results.** There was no significant main effect of ‘change’ on the amplitude of the S3-ERP (N2, P2: $p>0.05$). There was a significant main effect of posture in the P2 wave ($F=9.67$, $p<0.05$), but not in the N2 wave ($p>0.05$). Crucially, there was a significant interaction change × posture interaction (N2: $F=5.72$, $p<0.05$; P2: $p>0.05$), indicating that the dishabituation caused by a selective change in the spatial location of the stimulus only occurred in the far condition. **Conclusion.** Within a stream of identical nociceptive stimuli, heterosegmental changes in their location can strongly dishabituate the ERP response. Importantly, such dishabituation is dramatically dependent on the spatial distance between the stimuli in egocentric coordinates. This observation supports the notion that laser ERPs reflect the detection of behaviourally-relevant changes in the sensory environment.

Disclosures: M. Moayedi: None. **G. Di Stefano:** None. **G. Iannetti:** None.

Nanosymposium

390. Pain Imaging: From Neural Circuits to Perception

Location: 147B

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 390.04

Topic: D.08. Pain

Support: CIHR

Title: Functional connectivity in salience and sensorimotor brain networks relates to pain and fatigue in ankylosing spondylitis

Authors: *K. S. HEMINGTON[1,2], Q. WU[1], A. KUCYI[1,2], R. D. INMAN[1,3], K. D. DAVIS[1,4]; [1]Toronto Western Res. Inst., Univ. Hlth. Network, Toronto, ON, Canada; [2]Inst. of Med. Sci., [3]Med. and Inst. of Med. Sci., [4]Surgery and Inst. of Med. Sci., Univ. of Toronto, Toronto, ON, Canada

Abstract: Introduction: Ankylosing spondylitis (AS) is a spondyloarthritis of the axial skeleton that can contribute to debilitating fatigue, mobility restrictions and chronic back pain, especially at night. Recently, we reported grey matter abnormalities related to neuropathic pain in pain-related brain networks as well as fatigue-related gray and white matter abnormalities in attention and motor areas of the brain in AS (Wu et al., 2013; 2014). In chronic pain, functional connectivity between brain regions associated with pain intensity and salience often becomes disturbed but the relation of these abnormalities to clinical features are not well understood. Therefore, we explored whether the functional connectivity of salience and sensorimotor brain networks are impacted by the severity of pain and fatigue in patients with AS. Methods: Patients with AS (ages 22-60) and age-/sex-matched healthy controls (ages 25-59) provided informed written consent and participated in a resting state functional MRI session (five minutes), with scans acquired on a GE Signa 3T MRI scanner. Patients completed the Fatigue Severity Scale and measures of total back pain and nocturnal back pain (all on a 0-10 scale) were obtained via patient charts. Using a functional connectivity atlas, we defined seed-target region pairs to represent the salience network (anterior/mid cingulate cortex (ACC/MCC) and insula) and the sensorimotor network (primary sensorimotor areas (M1/S1) and supplementary motor area (SMA)) (Shirer et al., 2012). Functional connectivity analysis was performed using FSL and MATLAB, and the Fisher-transformed correlation between seed-region pairs was calculated. We then correlated resting state functional connectivity to the individual reports of AS average back pain, nocturnal back pain, and fatigue severity. Results: The severity of pain and fatigue varied across the AS group with average back pain ranging from 3-9, nocturnal back pain from 2-9, and fatigue from 1.7-10. Functional connectivity of both the salience network (ACC- insula), and the

sensorimotor network (S1/M1-SMA) strongly correlated (r>0.5) with the individual reports of average back pain, nocturnal back pain, and fatigue severity. There were no overall significant group differences in mean functional connectivity between patients and controls for any of the seed-region pairs. Conclusion: This study demonstrates that the strength of resting state functional connectivity within the salience and sensorimotor networks tracks the severity of pain and fatigue experienced in AS. These relationships may reflect the degree of plasticity induced by chronic pain and fatigue in AS.

Disclosures: K.S. Hemington: None. **Q. Wu:** None. **A. Kucyi:** None. **R.D. Inman:** None. **K.D. Davis:** None.

Nanosymposium

390. Pain Imaging: From Neural Circuits to Perception

Location: 147B

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 390.05

Topic: D.08. Pain

Support: Department of Neural and Pain Sciences, University of Maryland School of Dentistry

Title: Pain and cognitive neural processing in migraine and the influence of disease severity and pain catastrophizing

Authors: *V. A. MATHUR[1,2], S. A. KHAN[1], C. S. HUBBARD[1], M. L. KEASER[1], M. GOYAL[3], D. A. SEMINOWICZ[1];
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Abstract: Prior work suggests that migraine alters neural responses to acute pain stimuli, but whether these changes are associated with migraine severity or psychological responses to pain is unclear. Also, while abnormal cognitive network activity has been reported in other pain populations, little is known about the effect of migraine on these networks. Here, we used fMRI to examine pain and cognitive neural processing in migraine, and the influence of disease severity and pain catastrophizing on these processes. Fourteen chronic migraine patients (11f, mean age 40.8, range 18-59) and 14 healthy controls (11f, mean age 38.9, range 20-61) completed two 10min fMRI runs (Siemens 3T Tim-Trio, TR=2.5s, resolution = 1.8x1.8x4mm) where they received heat stimuli (three intensity levels, two painful) on the left forearm and performed a modified Attentional Network Test (two levels of task-difficulty) within a block

design. Subject-level GLMs were modulated by reaction time. All group-level results were RFT-based cluster corrected at $p<.05$. Patients self-reported migraine severity over the past month, and all participants completed the Pain Catastrophizing Scale. Pain-related activity was not significantly different between patients and controls ($p_{uncorr}>.005$). Among patients, pain-related activity in the bilateral anterior insula (aINS) was positively correlated with pain catastrophizing, and activity in the bilateral aINS and right posterior insula (pINS) was associated with migraine pain over the past month. Both pain catastrophizing and migraine pain were negatively associated with activity in default mode network (DMN) regions. Across groups, cognitive networks were recruited in response to the cognitive task, and pain-task interactions were found in the right pINS (increased with pain, decreased with task). Consistent with previous findings in other pain populations, migraine patients had less task-related deactivation within the left dorsolateral prefrontal cortex (dlPFC), left dorsal anterior cingulate cortex (dACC), right pINS, and left dorsal superior frontal gyrus compared to controls. Prior research has associated these regions with decreased cortical thickness in chronic pain populations as well as altered cognitive function and pain regulation. Our findings suggest that migraine-related changes in pain networks are related to clinical characteristics (migraine pain intensity) and psychological responses to pain (pain catastrophizing), and that migraine alters cognitive network functioning, particularly brain regions previously shown to be affected by chronic pain, and restored with effective pain treatment.

Disclosures: V.A. Mathur: None. **S.A. Khan:** None. **C.S. Hubbard:** None. **M.L. Keaser:** None. **M. Goyal:** None. **D.A. Seminowicz:** None.

Nanosymposium

390. Pain Imaging: From Neural Circuits to Perception

Location: 147B

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 390.06

Topic: D.08. Pain

Support: German Research Foundation (DFG), SFB 936/A5

Title: Expectations modulate long-term habituation and significantly influence connectivity between pain-processing areas in a standardized heat pain paradigm

Authors: *I. S. ELLERBROCK, A. MAY;
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Abstract: Habituation and sensitization are two major responses to repetitively administered pain and can be modulated by negative and positive expectations. Recently we designed a specific longitudinal nociceptive method where subjects robustly habituate between-session and sensitize within-session over a period of eight days (1). Changes in BOLD-signal accompanied these behavioral findings: habituation over days is mirrored by a decrease of activity in pain processing brain whereas context-specific expectations prompt an increase in the insular cortex (2). We extended this method using a 21 days repetitive stimulation and focused on brain areas which are functionally coupled with and without context modulation using psychophysiological interaction (PPI) analysis. A standardized longitudinal heat paradigm consisting of 60 suprathreshold (46°C) nociceptive stimuli was applied on 21 consecutive days to the volunteers' left volar forearm. 40 healthy participants were randomly assigned to two groups: one group was not given further information (control, n=20) whereas the other group received the information that pain perception will probably increase over time (nocebo, n=20). Perceived pain intensity was assessed on a 0-100 visual analog scale (VAS). On days 1, 8, 14, and 21, experimental testing was conducted during functional MRI scanning. Daily sensitization was observed in both groups throughout the complete study trial. Both groups habituated over time, however, significantly less in the nocebo group. Only the control reached a plateau around day 16, i.e. the pain ratings did not decrease any further. Decreased pain perception over days was reflected in reduced BOLD-signal in pain processing areas, such as insula and somatosensory cortices, whereas the activation in the rACC increased over time. Consistent with previous results, context manipulation was accompanied by activation of the operculum, which exhibited a stronger coupling to pain-processing areas in the nocebo group during nociceptive input. Additionally, significant changes in this network became apparent when comparing the first and last day of the study trial. Our findings highlight the importance of context information in experimental pain studies which may be effective even longer than 3 weeks and explore connectivity changes (3) between brain areas involved in pain processing as an important neuronal basis of this effect.
References: (1)May et al., Eur J Pain, 2012 (2)Rodriguez-Raecke et al., J Neurosci, 2010 (3)Ellerbrock & May

Disclosures: I.S. Ellerbrock: None. **A. May:** None.

Nanosymposium

390. Pain Imaging: From Neural Circuits to Perception

Location: 147B

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 390.07

Topic: D.08. Pain

Support: NIH Grant 1K01HD0695045

American Heart Association 13BGIA17120055

Title: Transcranial direct current stimulation: Modulating functional connectivity across pain networks

Authors: *V. SANKARASUBRAMANIAN[1], D. CUNNINGHAM[1], S. ROELLE[1], K. POTTER-BAKER[1], E. BEALL[2], A. MACHADO[3], E. PLOW[1,4];
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Abstract: Background: Chronic neuropathic pain is subtended by a complex circuitry, including sensory-discriminative and affective-emotional networks spanning several cortical and subcortical structures. Noninvasive brain stimulation is fast becoming popular as an investigational modality to affect pain experience. Stimulation, particularly anodal transcranial direct current stimulation (tDCS) applied to the primary motor (M1) or the dorsolateral prefontal cortices (DLPFC) represent two popular methods. However, it is still unclear how anodal tDCS of M1 versus DLPFC affects complex circuitry of pain, evidence that could provide better understanding as to whether they may affect varying aspects of pain experience. **Objective:** To investigate and compare the effects of anodal tDCS to M1 versus DLPFC upon functional connectivity (FC) across cortical-subcortical networks of pain. **Methods and Design:** Healthy subjects received either sham, anodal tDCS to M1 or anodal tDCS to DLPFC on three separate occasions. A seed-based FC analysis of the thalamus was conducted, and it's connectivity across cortical-subcortical pain networks were studied using resting state functional magnetic resonance imaging (rs-fMRI), before and after tDCS. Sham stimulation helped control for placebo or time-varying effects on FC. Activation maps of the M1 and DLPFC stimulation sessions were subtracted with the sham session to determine tDCS-specific changes. Finally, the validity of tDCS on pain versus non-pain networks was tested by measuring the effects of stimulation on the FC of thalamo-occipital networks. **Results:** Anodal tDCS to left M1 significantly increased the ipsilateral thalamic-M1 connectivity and decreased the coupling between ipsilateral thalamus and insula, as compared to sham. In contrast, anodal stimulation to left DLPFC significantly increased the FC of the ipsilateral thalamus with both the DLPFC and insula, but decreased ipsilateral thalamic-M1 connectivity. Thalamic-occipital connectivity was not modulated with any form of tDCS, validating the effect of tDCS on pain networks only. **Conclusions and future outlook:** Since anodal tDCS delivered to M1 and DLPFC modulate connectivity differently across cortical-subcortical networks of pain, our evidence supports claims about their varying effects on pain. This is beneficial and can affect clinical practice of pain especially, as now,

depending on the specificity of etiology, impairment level, and the duration of chronic neuropathic pain, patients may witness different responses to these two targets of stimulation.

Disclosures: V. Sankarasubramanian: None. **D. Cunningham:** None. **S. Roelle:** None. **K. Potter-Baker:** None. **E. Beall:** None. **A. Machado:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intelect Medical, ATI, Enspire, Cardionomics. F. Consulting Fees (e.g., advisory boards); Intelect Medical, Functional Neurostimulation, Deep Brain Innovations. **E. Plow:** None.

Nanosymposium

390. Pain Imaging: From Neural Circuits to Perception

Location: 147B

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 390.08

Topic: D.08. Pain

Title: Task-negative network dysfunction in fibromyalgia patients is related to lack of modulation by cognitive load

Authors: *M. CEKO[1], J. L. GRACELY[2], M.-A. FITZCHARLES[3], D. A. SEMINOWICZ[4], P. SCHWEINHARDT[3], M. BUSHNELL[2];
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Abstract: The task-negative network (TNN), including the posterior cingulate cortex (PCC) and medial prefrontal cortex (MPFC), shows consistently decreased activation (i.e. deactivation) during cognitive task performance in healthy individuals. The magnitude of TNN deactivation increases with cognitive load (Mayer et al., 2010, *Hum Brain Mapp*). In chronic pain patients, decreased deactivation of TNN has been reported during a cognitive task, however the effect of varying task difficulty is unknown. Here we examined the effect of cognitive load on TNN deactivation in fibromyalgia, a chronic pain syndrome associated with cognitive dysfunction. We compared 28 female fibromyalgia patients to 24 female age-matched controls (Ceko et al., 2013, *NeuroImage Clin*). Fibromyalgia patients had no co-morbid clinical depression or anxiety disorder, but showed significantly worse performance on a complex cognitive test (Auditory Consonant Trigram) (Ceko et al., 2013 *SFN Abstracts*). Here, groups were trained on the N-back working memory task (three levels of difficulty (1-,2-,3-back) compared to control level (0-back)) to achieve comparable behavioral performance (reaction time, accuracy; analyzed with ANOVA). Subjects then underwent a functional MRI while performing the N-back task (3T

Siemens Trio, EPI BOLD, 3.5 mm isotropic voxels). Functional MRI data were preprocessed and analyzed in AFNI (http://afni.nimh.nih.gov); 3dMEMA GLM was used for group comparisons contrasting each N-back level to 0-back. Results were considered significant at $p<0.01$ voxel-wise threshold, $p<0.05$ cluster-corrected with 3dClustSim across task-relevant brain regions (mask derived from meta-analysis of functional MRI studies of working memory). Both groups displayed worsening of performance as cognitive load increased (ANOVA, $p<0.001$), but groups did not significantly differ in their performance on any of the levels (ANOVA, $ps>0.2$). Consistent with previous studies, patients had decreased deactivation of the TNN compared to controls during cognitive task performance, most pronounced at 2-0 back (PCC, MPFC $p<0.05$ corrected). Importantly, while healthy controls showed increased deactivation of the TNN with increasing cognitive load, this modulation was absent in patients ($p<0.05$ group difference; ANOVA on fMRI signal in PCC). Our results suggest that impaired deactivation of the TNN during task performance in chronic pain patients is related to a failure to modulate this network by cognitive load.

Disclosures: **M. Ceko:** None. **J.L. Gracely:** None. **M. Fitzcharles:** None. **D.A. Seminowicz:** None. **P. Schweinhardt:** None. **M. Bushnell:** None.

Nanosymposium

390. Pain Imaging: From Neural Circuits to Perception

Location: 147B

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 390.09

Topic: D.08. Pain

Support: Pfizer Neuropathic Pain Award

Title: Assessing mean diffusivity in gray matter regions in a sample of fibromyalgia patients

Authors: ***N. FEIER**[1,2], M. CEKO[1,2,4], M. FITZCHARLES[3], P. SCHWEINHARDT[1,2]; [1]Dent., [2]Alan Edwards Ctr. for Res. on Pain, [3]Rheumatology, McGill Univ., Montreal, QC, Canada; [4]Natl. Ctr. for Complementary and Alternative Med., Natl. Inst. of Hlth., Bethesda, MD

Abstract: Fibromyalgia syndrome is a widespread pain disorder lacking detectable pathology making it difficult to diagnose and treat. In recent years, neuroimaging has been used to investigate morphological brain changes related to fibromyalgia. Magnetic resonance imaging (MRI) studies have revealed widespread gray matter (GM) decreases in a variety of brain regions thought to be involved in pain processing. A previous study from our group contributed to this

literature by reporting gray matter alterations in a sample of 28 female fibromyalgia patients compared to 28 matched controls using voxel-based morphometry (VBM). In the present study, we aim to examine the nature of these GM decreases by assessing mean diffusivity as a surrogate measure of tissue density in the same brain regions. Mean diffusivity is calculated from diffusion tensor images (DTI) and represents a measure of average water diffusion, most commonly examined in white matter. We adapted the processing pipelines, mainly the registration of diffusion and anatomical images, for the investigation of gray matter. Multiple registration techniques were tested and a non-linear registration method was selected that provided the most accurate registration. We hypothesize that mean diffusivity will be increased in regions where we found GM decreases, which would indicate that volumetric changes as measured with VBM are a consequence of decreased tissue density. We expect mean diffusivity to be slightly increased in areas where GM decreases have been reported in other fibromyalgia studies, which would suggest that diffusion measurements are more sensitive measures for GM alterations than volumetric measurements. We expect no change in mean diffusivity measures in areas where there have been no previously reported GM alterations. Confirmation of our hypotheses might indicate that decreased tissue density in fibromyalgia is a consequence of sustained input via ascending pain pathways leading to eventual cell shrinkage and perhaps even cell death in pain processing regions. Examining the tissue density underlying volumetric changes in fibromyalgia brings us one step closer to understanding the mechanisms involved in this complex pain disorder.

Disclosures: **N. Feier:** None. **M. Ceko:** None. **M. Fitzcharles:** None. **P. Schweinhardt:** None.

Nanosymposium

390. Pain Imaging: From Neural Circuits to Perception

Location: 147B

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 390.10

Topic: D.08. Pain

Support: 094863/Z/10/Z

Title: Obligatory components of laser-evoked potentials

Authors: ***F. MANCINI**[1], G. DI STEFANO[2], A. MOURAUX[3], G. IANNETTI[4];
[1]UCL, London, United Kingdom; [2]Universita' La Sapienza, Roma, Italy; [3]Univ. Catholique de Louvain, Brussels, Belgium; [4]Univ. Col. London, London, United Kingdom

Abstract: The recording of transient laser-evoked potentials (LEPs) from the ongoing EEG is the most established neurophysiological technique to assess the function of the nociceptive system in humans. LEPs are typically recorded using long and unpredictable inter-stimulus intervals (e.g. 5-10 seconds). However, these stimulation paradigms make the LEPs magnitude strongly determined by unspecific stimulus-triggered attentional processing (Iannetti et al., 2008; Mouraux and Iannetti 2009), and thus not optimal to study nociceptive-specific neural activity (Mouraux et al., 2013). We recorded LEPs in 15 healthy volunteers. Laser pulses were delivered at 1 Hz, in blocks of 60 stimuli, on the dorsum of either the right or left hand. 1-Hz laser stimulation evoked a clear LEP response, albeit of smaller magnitude compared to the LEP elicited by long and unpredictable intervals. Notably, the LEP comprised a clear negative response (N1 wave), originating from the contralateral primary sensorimotor cortex. The N1 component of LEPs did not habituate across the 60 stimuli delivered in each block. These findings suggest that the primary sensorimotor cortex is obligatorily involved in responding to nociceptive input, even when the stimulus saliency is drastically reduced. LEPs elicited by high-frequency nociceptive stimulation provide a more direct readout of the state of nociceptive pathways than traditional methods, with potential clinical applications.

Disclosures: F. Mancini: None. **G. Di Stefano:** None. **A. Mouraux:** None. **G. Iannetti:** None.

Nanosymposium

390. Pain Imaging: From Neural Circuits to Perception

Location: 147B

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 390.11

Topic: D.08. Pain

Support: IIT from Eli Lilly

Title: Duloxetine and placebo treatments induce region-specific modification of gray matter density in osteoarthritis pain patients

Authors: *P. TÉTREAULT[1], M. N. BALIKI[1], É. VACHON-PRESSEAU[1], H. HISSAIN[1], M. FARMER[1], A. T. BARIA[1], T. J. SCHNITZER[2], A. V. APKARIAN[3];
[1]Physiol., [2]Rheumatology, [3]Anaesthesia and Surgery, Northwestern Univ., Chicago, IL

Abstract: Multiple structural neuroimaging studies show chronic pain is associated with decreases in cortical and subcortical gray matter density across different patient cohorts. However the extent and pattern of reversibility of these changes with successful intervention

remains largely unknown. In this study, we evaluate structural brain reorganization in patients with chronic knee osteoarthritis (OA) pain after four months of treatment with duloxetine (DLX) or placebo. The main aim of the study was to assess whether anatomical abnormalities were reversible and dependent on treatment outcomes Patients with chronic OA pain (n=40) were recruited and entered into a placebo-controlled double blind study. Participants underwent one initial anatomical MRI scan using a 3T Siemens scanner. An identical scanning session was performed at the end of the treatment (i.e.: 30 mg/day (1 week), 60 mg/day (14 weeks) and taper down to 30 mg/day (last week)). Patients were randomized to receive either DLX or placebo, and were further divided after treatment into responders (≥ 20% in pain decrease) and non-responders. Changes in cortical and subcortical regions gray matter properties were assessed using voxel based morphometry (FSL-VBM) and model-based segmentation tool (FIRST). Patients treated with DLX did not show any significant pain decrease compared to placebo ($p > 0.05$, repeated measure ANOVA). A two-way repeated measure ANOVA was utilized to investigate changes in whole-brain grey matter properties in relation to treatment, response, and their interaction. Subcortical regions including the right amygdala and nucleus accumbens showed significant response effect ($p<0.01$; regardless of treatment), both were significantly larger in the responders. Neocortical gray matter density exhibited localized changes for treatment, response, and for treatment x response effects. Patients that exhibited significant decrease in pain showed increased gray matter density in primary somatosensory cortex. On the other hand, DLX-treated patients showed decreased grey matter density in bilateral ACC, left lateral post-central gyrus. Finally, successful treatment with DLX was associated with increased gray matter density of the prefrontal cortex. These results show that pharmacological pain treatment affects mainly cortical reorganization rather than subcortical structures, and treatment type impacts distinct cortical regions. On the other hand differences observed before the beginning of the treatment in specific subcortical regions between responders and non-responders suggest that these structures may be used as a predictors for treatment response.

Disclosures: P. Tétreault: None. **M.N. Baliki:** None. **É. Vachon-Presseau:** None. **H. Hissain:** None. **M. Farmer:** None. **A.T. Baria:** None. **T.J. Schnitzer:** None. **A.V. Apkarian:** 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.; IIT from Eli Lilly.

Nanosymposium

390. Pain Imaging: From Neural Circuits to Perception

Location: 147B

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 390.12

Topic: D.08. Pain

Support: Pfizer Neuropathic Pain Award

BC Epilepsy Society

Canadian Institutes of Health Research

Title: Pharmacological analysis of cortical spreading depression in familial hemiplegic migraine type-1 mice

Authors: *S. M. CAIN[1,2], B. BOHNET[3], H. HAN[2], A. C. YUNG[3], P. KOZLOWSKI[3], B. A. MACVICAR[2], T. P. SNUTCH[1,2];
[1]Michael Smith Labs., [2]Ctr. for Brain Hlth., [3]7T MRI Facility, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Patients with Familial Hemiplegic Migraine Type-1 (FHM-1) suffer from migraine, sometimes including ataxia and seizures, resulting from gain-of-function mutations in the Cav2.1 (P/Q-type) calcium channel. Genetic knock-in mice with FHM-1 mutations (van den Maagdenberg et al., 2004, 2010) display a reduced threshold for Cortical Spreading Depression (CSD), which is believed to be the underlying pathophysiological trigger of migraine headaches. We have used intrinsic optical signalling to examine CSD in vitro using acute brain slices from wild-type and FHM-1 mice. In addition, we have correlated these findings with electrophysiological analysis of synaptic activity using whole-cell patch clamp in acute brain slices. Furthermore, we have utilized 7 Telsa magnetic resonance imaging to visualize the speed and threshold of CSD in vivo at high spatial and time resolution, providing a clear map of the structures involved across the brain. Finally, we have examined clinically available pharmacological agents to determine whether these are capable of either slowing, or increasing the threshold for CSD. Our findings indicate that both CSD speed and threshold can be affected to different degrees by pharmacological drug action in wild-type versus FHM-1 mutant mice. They also identity specific brain regions affected by FHM-1 mutations in the mouse models.
References Van den Maagdenberg AM et al. (2010) High cortical spreading depression susceptibility and migraine-associated symptoms in Ca(v)2.1 S218L mice. Ann Neurol 67:85-98. Van den Maagdenberg AM, Pietrobon D, Pizzorusso T, Kaja S, Broos LA, Cesetti T, van de Ven RC, Tottene A, van der Kaa J, Plomp JJ, Frants RR, Ferrari MD (2004) A Cacna1a knockin migraine mouse model with increased susceptibility to cortical spreading depression. Neuron 41:701-710.

Disclosures: **S.M. Cain:** None. **B. Bohnet:** None. **H. Han:** None. **A.C. Yung:** None. **P. Kozlowski:** None. **B.A. MacVicar:** None. **T.P. Snutch:** None.

Nanosymposium

391. Cellular Effects of Stress

Location: 146C

Time: Monday, November 17, 2014, 1:00 PM - 3:45 PM

Presentation Number: 391.01

Topic: E.05. Stress and the Brain

Support: US army grant DM102281

Title: Early intervention with intranasal neuropeptide prevents single prolonged stress triggered neuroendocrine impairments in hypothalamus and ventral hippocampus

Authors: M. LAUKOVA[1], L. G. ALALUF[1], L. I. SEROVA[1], V. ARANGO[2], *E. L. SABBAN[1];
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Abstract: Intranasal administration of neuropeptide Y (NPY) is a promising treatment strategy to reduce traumatic stress-induced behavioral symptoms related to posttraumatic stress disorder (PTSD)(Serova et. al., 2013, Neurosci, 236, 298-312). Molecular mechanisms underlying beneficial effects of NPY on stress-induced impairments in hypothalamic-pituitary-adrenocortical (HPA) axis and ventral hippocampus was studied using single prolonged stress (SPS) as an animal model of PTSD. Immediately after exposure to the SPS stressors, Sprague Dawley male rats were infused with 150 μg of NPY or vehicle and sacrificed after 7 days undisturbed period. Plasma ACTH and corticosterone, and hypothalamic corticotropin-releasing hormone (CRH) mRNA were significantly higher in the vehicle, but not in the NPY-treated group, compared to unstressed controls. Although total glucocorticoid receptor (GR) protein levels were not altered in mediobasal hypothalamus, a significant decrease of GR phosphorylated form on serine 232 and increased FKBP5 mRNA were found with the vehicle, but not in animals infused with intranasal NPY. Immunofluorescence of CRH was lower in mediobasal hypothalamus of both SPS-treated groups compared to unstressed controls. In the ventral hippocampus, only vehicle-treated animals demonstrated elevated GR protein expression and increased GR phosphorylation on Ser232, specifically in the nuclear fraction. Additionally, SPS-induced increase of CRH mRNA in the ventral hippocampus was accompanied by a decrease of CRH peptide particularly in the CA3 subfield, both prevented by NPY. The results show that early intervention with intranasal NPY can prevent traumatic stress-triggered dysregulation of the HPA axis likely by restoring proper negative feedback inhibition of the HPA axis via functional GR. Thus, intranasal NPY has a potential as a non-invasive therapy to prevent negative effects of traumatic stress.

Disclosures: **M. Laukova:** None. **E.L. Sabban:** None. **L.G. Alaluf:** None. **L.I. Serova:** None. **V. Arango:** None.

Nanosymposium

391. Cellular Effects of Stress

Location: 146C

Time: Monday, November 17, 2014, 1:00 PM - 3:45 PM

Presentation Number: 391.02

Topic: E.05. Stress and the Brain

Support: NIH Grant NS078434

Title: Characterization of specific neuronal types in the bed nucleus of the stria terminalis aided by using multiple transgenic mouse lines

Authors: A. Q. NGUYEN[1], *X. XU[2];
[1]Anat. and Neurobio., Univ. of California, Irvine, Irvine, CA; [2]Anat. and Neurobio., Univ. California, Irvine, Irvine, CA

Abstract: The bed nucleus of the stria terminali (BNST) plays an important role in fear, stress, and anxiety. The BNST comprises a collection of nuclei typically delineated by gross cytoarchitecture features, but the neurochemical makeup of the BNST neurons has yet to be well characterized. In this study, we use transgenic mice lines expressing specific chemical markers in conjunction with immunochemical staining to characterize BNST cell types. The mouse lines include GAD67-GFP mice and specific Cre driven lines - GAD2-Cre, CaMKIIα-Cre, VGLUT2-Cre, ChAT-Cre, CRH-Cre, PV-Cre, SOM-Cre, CR-Cre, and VIP-Cre - which are crossed to a red fluorescent reporter line (Ai9). We first determined the distributions of glutamatergic excitatory and GABAergic inhibitory neurons using immunostaining of excitatory amino acid carrier 1 (EAAC1) in double transgenic mice of GAD2-Cre:Ai9 and GAD67-GFP. The densities of excitatory neurons are 31.5 ± 3.0 cells/mm2 and 55 ± 6.5 cells/mm2 for dorsal and ventral regions of anterior BNST sections, respectively, and the inhibitory neuronal densities are 152.6 ± 8.8 cells/mm2 and 62.6 ± 3.8 cells/mm2 for the two regions. A great majority of BNST cells are GABAergic inhibitory neurons as 70% of dorsal, and 65% of ventral BNST neurons are comprised of GAD+ neurons. Excitatory neurons determined by EAAC1 staining account for 14% in dorsal and 32% in ventral regions. Measurements from CaMKIIα-Cre:Ai9 and VGLUT2-Cre:Ai9 sections indicated that 14% dorsal and 8% ventral BNST neurons are comprised of CaMKIIα+ cells, and less than 3% BNST neurons are VGLUT2+ . Notably, compared to the uniform distribution of GAD67+ cells, the concentrated GAD2+ cells can be used to delineate

the juxtacapsular (JC) subregion. Among the sub-types of GABAergic cells, there are no or few parvalbumin (PV)+ cells in BNST. Somatostatin (SOM)+ cells accounts for 18% and 5% BNST cells in dorsal and ventral regions, respectively, while calretinin (CR)+ cells make up 10% and 7% cells in these two regions. Despite sparse vasoactive intestinal peptide (VIP)+ neuronal cell bodies, VIP+ axonal plexuses are found prominently in the anterolateral region containing both the oval and JC subregions. Corticotropin-releasing hormone (CRH)+ cells have average densities of 18.7 ± 2.1 cells/mm2 and 25.29 ± 1.97 cells/mm2 in dorsal and ventral BNST. There are few ChAT+ cells with the average density being < 3 cell/mm2, and appeared to be concentrated in the JC subregion. This work provides new information on BNST neuronal components and sub-regional delineation, and can help future studies of specific inhibitory and excitatory neurons in the BNST.

Disclosures: A.Q. Nguyen: None. **X. Xu:** None.

Nanosymposium

391. Cellular Effects of Stress

Location: 146C

Time: Monday, November 17, 2014, 1:00 PM - 3:45 PM

Presentation Number: 391.03

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIDA DA032280

AFSP SRG-1-135-11

Title: Biochemical and genetic evidence of the role of Akt signaling in fear memory processing and depression-like behaviors

Authors: *T. F. FRANKE;
Psychiatry, Pharmacol. and Mol. Biochem., NYU Sch. of Med., New York, NY

Abstract: Background: The Akt/PKB family of serine/threonine kinases plays a key role in neuronal cell function. Mutations in human AKT genes are found in human schizophrenic subjects, and other neurodevelopmental and psychiatric disorders. Genes encoding the three Akt isoforms (Akt1, Akt2, Akt3) are expressed in the mouse brain suggesting that Akt-mutant mice are suitable models to better understand the consequences of AKT gene dysfunction in the human brain. Methods: To model the impact of Akt genotypes on fear memory processing and depression-like behaviors, we used genomic knockout strains for individual mouse Akt genes

(Akt1, Akt2, Akt3) to examine genotype-dependent behavioral responsiveness and biochemical phenotypes. Results: Mice deficient for single Akt genes exhibited isoform-specific behavioral phenotypes during the acquisition and subsequent processing of fear memories. To complement our results in Akt-mutant mice with a pharmacological approach, we inhibited Akt with an allosteric inhibitor prior to testing. Baseline anxiety levels in naive Akt-mutant mice or wild-type littermates with inhibited Akt signaling were not altered. Acute stress exposure of Akt-mutant or inhibitor-treated mice resulted in increased depression-like behavioral responsiveness. During and after chronic stress in the social defeat paradigm, Akt knockout mice exhibited an increased susceptibility to social defeat and decreased responsiveness to chronic antidepressant treatment. Discussion: Our data in Akt-mutant mice converge with genetic findings in human subjects to support a critical role for Akt signaling in cognition and mood regulation. The importance of intact Akt signaling for resilience to chronic stress is predicted from prior studies using overexpression of virus encoding mutant Akt proteins. Still, our current findings define the physiological contribution of selected Akt kinase isoforms to fear memory processing and the resilience to chronic stress. Our results suggest the utility of specific Akt kinase isoforms as novel diagnostic markers and therapeutic targets for the treatment of neurodevelopmental and psychiatric disorders.

Disclosures: T.F. Franke: None.

Nanosymposium

391. Cellular Effects of Stress

Location: 146C

Time: Monday, November 17, 2014, 1:00 PM - 3:45 PM

Presentation Number: 391.04

Topic: E.05. Stress and the Brain

Support: NIH Grant NS073899

Title: Tau and FKBP51 cause depressive-like symptoms by regulating glucocorticoid signaling

Authors: *J. J. SABBAGH, J. C. O'LEARY, III, L. J. BLAIR, S. N. FONTAINE, C. A. DICKEY;
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Abstract: FK506 binding protein 51 (FKBP51), encoded by the FKBP5 gene, is an Hsp90 co-chaperone implicated in multiple psychiatric diseases, including depression and Alzheimer's disease (AD). FKBP51 levels increase with age due to reduced FKBP5 methylation, leading to

vulnerability to stress and altered glucocorticoid signaling. FKBP5-/- mice display anti-depressive behavior, resilience to stress-induced neuroendocrine activity, and decreased tau levels. The microtubule motor dynein has already been linked to GR nuclear translocation, suggesting a possible role for the microtubule-associated protein tau in this process as well. We sought to determine the functional and mechanistic connections between tau and FKBP51 by investigating how they regulate GR activity in the brain. FKBP5-/- mice demonstrated an anti-depressive phenotype across the lifespan and a concomitant decrease in stress-induced serum corticosterone (CORT) levels. Acute exogenous CORT rescued this phenotype suggesting FKBP51 regulates depressive behavior through a glucocorticoid-dependent mechanism. GR nuclear translocation was enhanced via immunofluorescent microscopy and ex vivo slices of FKBP5-/- mice. Preliminary evidence suggests tau also directly regulates GR activity and depression-like behavior, implicating tau in stress-related diseases. Specifically, tau deletion mimics the anti-depressive phenotype of FKBP5-/- mice, while manipulating tau expression alters GR activity. Thus tau and FKBP51 are necessary components of an intricate mechanism that slows GR activity and prolongs the systemic stress response.

Disclosures: J.J. Sabbagh: None. **J.C. O'Leary:** None. **L.J. Blair:** None. **S.N. Fontaine:** None. **C.A. Dickey:** None.

Nanosymposium

391. Cellular Effects of Stress

Location: 146C

Time: Monday, November 17, 2014, 1:00 PM - 3:45 PM

Presentation Number: 391.05

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: P30 GM103328

UMMC Intramural Research Support Grant

Title: KDM1A inhibition as an epigenetic priming strategy for attenuation of stressful memories

Authors: *M. RIAZ[1,2], M. BOHLEN[2], L. GOLDEN[1], I. A. PAUL[1], V. DURIC[3], R. S. DUMAN[3], C. A. STOCKMEIER[1];
[1]Psychiatry & Human Behavior, [2]Program in Neurosci., Univ. of Mississippi Med. Ctr., Jackson, MS; [3]Psychiatry, Yale Univ., New Haven, CT

Abstract: Depressive and stressor-related disorders represent the largest spectra of currently prevalent psychiatric illnesses and include Major Depressive Disorder (MDD) and Posttraumatic Stress Disorder (PTSD). Increasing evidence suggests that extinction learning of traumatic memories is epigenetically disrupted in these patients, with direct involvement of hippocampal-dependent learning & memory system. In fact, targeted inhibition of histone-modifying enzymes (HDAC1/2) has recently been demonstrated as an epigenetic priming strategy for attenuation of remote traumatic memories (Graff et al., 2014). In a mechanistic human postmortem study examining whole human transcriptome expression of hippocampal sub-regions, we noted that a novel epigene KDM1A was 10-fold up-regulated in MDD subjects (Duric et al., 2010). KDM1A is a lysine specific demethylase and works in conjunction with HDAC2 through the RCOR/GFI/KDM1A/HDAC nucleosomal complex regulating the epigenetic control of transcription by targeting chromatin lysine residues. We tested whether targeted inhibition of KDM1A will also attenuate remote stressor-related memories by employing a rodent model of unpredictable chronic mild stress (CMS) that has been validated to mimic a depressive-like phenotype. Male rats were first trained on hippocampal-dependent learning and memory tasks on an 8-arm radial arm maze, followed by exposure to CMS. Weekly measurements of reference memory performance were recorded to assess the expected cognitive decline followed by treatment with a novel brain-penetrant inhibitor of KDM1A activity (Neelamegam et al., 2013). A single dose of the KDM1A inhibitor (RN1, 10mg/kg ip) profoundly attenuated reference memories in both stressed and control rats. The reversion of both animal groups to the pre-stress performance level highlights the role of epigenetic switching in retrieval and reconsolidation of stressor memories. Our study supports the novel approach of epigenetic targeting for treatment of depressive and stressor-related disorders. Supported by P30 GM103328 and Univ. Mississippi Medical Center Intramural Research Support Grant.

Disclosures: **M. Riaz:** None. **M. Bohlen:** None. **L. Golden:** None. **I.A. Paul:** None. **V. Duric:** None. **R.S. Duman:** None. **C.A. Stockmeier:** None.

Nanosymposium

391. Cellular Effects of Stress

Location: 146C

Time: Monday, November 17, 2014, 1:00 PM - 3:45 PM

Presentation Number: 391.06

Topic: E.05. Stress and the Brain

Support: NSERC RGPIN 238950-2012

Title: Deficiency of CREB3/Luman results in maternal behavioral defects and stress/anxiety-related behavioral disorders in mice: a role of Luman in glucocorticoid signaling and neural protection

Authors: J. PENNEY, K. TRAN, M. ZENG, T. AMOR, J. LYMER, A. CARUSO, A. MCCLUGGAGE, P. TURNER, N. MACLUSKY, E. CHOLERIS, *R. LU;
Mol. and Cell. Biol., Univ. of Guelph, Guelph, ON, Canada

Abstract: The hypothalamic-pituitary-adrenal (HPA) axis is a major branch of the neuroendocrine system that regulates animal stress responses. Luman/CREB3 is a transcription factor that is involved in the unfolded protein response (UPR), while Luman-recruitment factor (LRF/CREBRF) is a regulator of Luman. Previously we have reported a maternal behavioral phenotype and dysregulation of glucocorticoid signaling in the LRF gene knockout (KO) mice. Here we report that Luman gene knockout mice have similar but more severe behavioral defects than the LRF KO mice, including maternal instinct deficit, stress resilience and hyperactivity. In an age-matched study, all pups born to Luman null mothers died within 2 days after birth due to maternal neglect, although these mutant females showed normal olfactory function and mammary gland development. Luman was found highly expressed in neuroendocrine tissues and pyramidal neurons in hippocampus cerebral cortex, critical for cognitive functions. As with LRF mice, glucocorticoid receptor signaling was impaired in the Luman null mice. At the cellular level, both Luman and LRF KO cells displayed altered cell proliferation properties, and heightened sensitivity to endoplasmic reticulum/Golgi stress and tendency for apoptosis. Overexpression of Luman significantly induced transcription from a glucocorticoid-responsive MMTV promoter. We propose that Luman, along with LRF, may represent a key regulatory circuit of the glucocorticoid signaling pathway, and plays a critical role in neural survival and the development of associated cognitive functions.

Disclosures: J. Penney: None. **K. Tran:** None. **M. Zeng:** None. **T. Amor:** None. **J. Lymer:** None. **A. McCluggage:** None. **P. Turner:** None. **N. MacLusky:** None. **E. Choleris:** None. **R. Lu:** None. **A. Caruso:** None.

Nanosymposium

391. Cellular Effects of Stress

Location: 146C

Time: Monday, November 17, 2014, 1:00 PM - 3:45 PM

Presentation Number: 391.07

Topic: E.05. Stress and the Brain

Support: Startup award to RK Leak

Title: Adaptation or sensitization to proteotoxic stress is dependent upon changes in glutathione

Authors: *R. K. LEAK, A. M. GLEIXNER, A. S. UNNITHAN, H. J. H. CHOI, J. WEILNAU; Pharmacol., Duquesne Univ., Pittsburgh, PA

Abstract: Many decades ago, Hans Selye proposed that stress could have both destructive and beneficial effects on animals. Chronic stress was deemed toxic, whereas mild stress (eustress) was thought to elicit compensatory adaptations that prepare the animal for the next challenge. Far less is known about how cells adapt to and survive severe stress, or stress that is intense enough to kill some fraction of the cellular population. Most authors studying severe stress only hypothesize that it weakens defenses in the surviving cells and exacerbates toxic responses to 2nd hits. For example, dual hits of severe stress can be synergistic in their toxic effects. We have collected evidence of this stress exacerbation in neuronal N2a cells treated with two sequential hits of the proteasome inhibitor MG132. Severely stressed cells exhibited catastrophic loss of glutathione and glutathione replacement abolished the synergistic response to dual proteotoxic hits of MG132. However, we have also gathered evidence in favor of adaptation to severe proteotoxic stress in primary cortical astrocytes. Severely stressed astrocytes that survived an initial hit failed to respond to a 2nd hit with additional cell loss, according to two independent viability assays. Stressed astrocytes increased production of ATP, glutathione, and heat shock proteins such as Hsp70 and Hsp32. Inhibition of Hsp70 activity did not abolish the resistance of stressed astrocytes to the 2nd hit. However, inhibition of glutathione synthesis did render stressed astrocytes vulnerable to the 2nd hit. Pre-stressed astrocytes also responded to the 2nd hit with a large increase in ubiquitinated proteins, demonstrating that we were not simply selecting for cells that were refractory to the MG132 poison. Thus, while some cells respond to severe stress with synergistic responses, others demonstrate much more resistance. Furthermore, high glutathione levels are linked to stress adaptation whereas low glutathione levels are linked to stress exacerbation. If similar stress adaptations are mounted in the human brain, they may explain why neurodegenerative disorders are so delayed in onset and so slow to progress. In effect, some types of cells may become progressively harder to kill. We are next contrasting the effect of dual hits in primary astrocytes harvested from neocortex to astrocytes harvested from neighboring allocortex. The neocortex is much less vulnerable than the allocortex to tau and alpha-synuclein inclusions in Alzheimer's and Parkinson's disease and we hypothesize that astrocyte heterogeneity in stress resistance may help explain the topographic appearance of protein inclusions in the telencephalon.

Disclosures: R.K. Leak: None. **A.M. Gleixner:** None. **A.S. Unnithan:** None. **H.J.H. Choi:** None. **J. Weilnau:** None.

Nanosymposium

391. Cellular Effects of Stress

Location: 146C

Time: Monday, November 17, 2014, 1:00 PM - 3:45 PM

Presentation Number: 391.08

Topic: E.05. Stress and the Brain

Support: American Heart Association Award 12POST1209001

Totman Medical Research Trust

Foundation Leducq

NIH Grant P20-RR-16435

NIH Grant P01-HL-095488

NIH Grant RO1-HL-044455

NIH Grant RO1-HL-098243

Title: Stress-induced glucocorticoid signaling remodels neurovascular coupling through impairment of cerebrovascular inwardly rectifying potassium channel function

Authors: T. A. LONGDEN[1], F. DABERTRAND[1], *S. E. HAMMACK[2], M. T. NELSON[1]; [1]Pharmacol., Univ. of Vermont Col. of Med., Burlington, VT; [2]Psychology, Univ. of Vermont, BURLINGTON, VT

Abstract: In the brain, oxygen and glucose are taken up from the blood on an 'as-needed' basis, to support ongoing neuronal function. To ensure adequate delivery of these nutrients, signaling cascades are initiated in response to an increase in neuronal activity, to evoke vasodilation of nearby parenchymal arterioles (PAs) and increase blood flow to the active tissue_this process is termed neurovascular coupling (NVC). One mechanism underlying NVC involves an increase in astrocytic intracellular Ca^{2+} driven by local neuronal activity, which triggers the local release of vasodilatory potassium (K^+) ions from specialized astrocytic endfoot processes at the gliovascular interface. These K^+ ions activate inwardly rectifying K^+ (K_{IR}) channels on the vascular smooth muscle, leading to vasorelaxation and increased blood flow. Here we report that chronic heterotypical stressor exposure impairs NVC in the region of the amygdala. Male rats were administered a 7-dheterotypical stress paradigm, which produced anxiety-like behavioral changes. NVC was modeled by measuring PA vasodilation in response to neuronal stimulation in brain slices containing the amygdala. After stress, vasodilation of PAs to neuronal stimulation

was greatly reduced, and isolated PA responses to external K^+ were diminished, suggesting a defect in smooth muscle K_{IR} channel function. Consistent with these observations, stress caused a reduction in PA $K_{IR}2.1$ mRNA and in smooth muscle K_{IR} current density, and blocking KIR channels significantly inhibited NVC in control, but not in stressed, slices. Delivery of corticosterone for 7 d (without stressors) or RU486 (before stressors) mimicked and abrogated NVC impairment by stress, respectively. We conclude that stress causes a glucocorticoid receptor-mediated decrease in functional K_{IR} channels in amygdala PA smooth muscle. This renders arterioles less responsive to K^+ released from astrocytic endfeet during NVC, leading to impairment of this process. Because the fidelity of NVC is essential for neuronal health, the impairment characterized here may contribute to the pathophysiology of brain disorders with a stress component.

Disclosures: T.A. Longden: None. **F. Dabertrand:** None. **S.E. Hammack:** None. **M.T. Nelson:** None.

Nanosymposium

391. Cellular Effects of Stress

Location: 146C

Time: Monday, November 17, 2014, 1:00 PM - 3:45 PM

Presentation Number: 391.09

Topic: E.05. Stress and the Brain

Support: Ellison Medical Foundation

Title: Optogenetic stimulation of serotonergic neurons induces chaperone upregulation in distal tissues of Caenorhabditis elegans

Authors: *V. PRAHLAD[1], M. TATUM[1], M. R. CHIKKA[1], L. A. MARTINEZ-VELAZQUEZ[2], L. CHAUVE[3], R. I. MORIMOTO[3];
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Abstract: Organisms have evolved multiple responses to hostile conditions that are coordinated to enhance the probability of survival. All cells possess quality control mechanisms to protect their proteome from adverse conditions. In addition, many organisms have neuronal circuitry to sense and anticipate noxious stimuli. Here we show that in C. elegans neurosensory perception of stress can protect cellular protein homeostasis through regulated serotonin release. We found that

in this organism acute heat shock resulted in serotonin release within minutes of temperature increase. This release, stimulated by the optogenetic activation of serotonergic neurons alone was sufficient to activate HSF1 and increase chaperone expression in the absence of proteotoxic damage. Consequently, the optogenetic stimulation of serotonergic neurons was sufficient to non-autonomously suppress protein misfolding in body wall muscle cells in C. elegans models of protein aggregation. The ability to preemptively activate HSF1 and chaperone gene expression in anticipation of proteotoxic danger through the modulation of the serotonergic system could have powerful implications for the treatment of protein conformation diseases.

Disclosures: V. Prahlad: None. **M. Tatum:** None. **M.R. Chikka:** None. **L.A. Martinez-Velazquez:** None. **L. Chauve:** None. **R.I. Morimoto:** None.

Nanosymposium

391. Cellular Effects of Stress

Location: 146C

Time: Monday, November 17, 2014, 1:00 PM - 3:45 PM

Presentation Number: 391.10

Topic: E.05. Stress and the Brain

Support: NHMRC APP1028183

The Hillcrest Foundation

Title: Changes to the proteome of the adrenal medulla evoked 20 min to 24 h after a single episode of glucoprivation

Authors: *A. K. GOODCHILD[1], P. BOKINIEC[1], S. F. HASSAN[1], L. M. PARKER[1], R. VANDER WALL[1], P. HAYNES[2], M. Z. MOGHADDAM[3], M. MIRZAEI[1];
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Abstract: The response to glucoprivation or hypoglycaemia includes activation of adrenergic chromaffin cells of the adrenal medulla via splanchnic sympathetic nerve activation and the action of corticosterone. This counter regulatory response is impaired in Type 1 and Type 2 diabetes. Our aim was to determine the proteomic changes that are evoked in the adrenal medulla at five time points after the administration of 2-deoxy-D-glucose (2DG). 18 male Sprague Dawley rats received 2DG (400mg /kg ip) and were then anaesthetised (sodium pentobarbitone 100mg/kg) immediately at time 0min or at 20min, 50min, 4h, 8h, or 24h later (n=3 per group).

The adrenal glands were removed immediately and placed in ice cold media and the adrenal medulla extracted and frozen. Extracted proteins were then fractionated on SDS-PAGE gels, cut into nine pieces and in-gel digested with trypsin. Extracted peptides were separated on nano-flow liquid tandem mass spectrometry (nano-LC-MS/MS, LTQ-XL ion trap). Spectra were searched against rattus norvegicus protein sequence database using GPM software. Normalised spectral abundance factors (NSAFs) from biological triplicate analysis are used to perform quantitative analysis. Identified proteins were analyzed using R and Ingenuity Pathway Analysis to determine differential protein expression and their interactions in various pathways. The number of proteins identified reproducibly in each condition ranged from 981 for 0 min (control) to 984 for 20 min and 819 for 8hr after drug injection. When compared to control using Student's t-test analysis, 78 and 165 proteins were differentially expressed 20min and 4h rats after receiving 2DG representing the smallest and largest changes respectively seen in the 5 time points analysed. Analysis of protein function demonstrate at 20min changes are associated with translational processes. Signal processes are significantly changed at 4h as VAT1 is upregulated 5.2 fold. VAT1 is an important vesicle related protein found in cholinergic terminals. This was associated with significant changes in a number of other signalling and vesicle related proteins. Even at 24 hours several more proteins were up regulated than at 20 min. One of these proteins was tyrosine hydroxylase, the rate limiting enzyme in catecholamine synthesis, which was upregulated by 2.9 fold using MS and 2.4 fold following Western Blot analysis of the same sample. A full functional analysis will be presented. This study has implications for the time course of response to a single insult as well as identification of the cellular pathways and processes changed under conditions of acute glucoprivation.

Disclosures: **A.K. Goodchild:** None. **P. Bokiniec:** None. **S.F. Hassan:** None. **L.M. Parker:** None. **R. Vander Wall:** None. **P. Haynes:** None. **M. Mirzaei:** None. **M.Z. Moghaddam:** None.

Nanosymposium

391. Cellular Effects of Stress

Location: 146C

Time: Monday, November 17, 2014, 1:00 PM - 3:45 PM

Presentation Number: 391.11

Topic: E.05. Stress and the Brain

Support: NIH Grant 1SC2GM105487

Title: Cellular stress induces a protective sleep response in *C. elegans*

Authors: *A. J. HILL, J. M. N. G. LOPEZ, R. MANSFIELD, C. VAN BUSKIRK;
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Abstract: Despite widespread recognition of the importance of sleep, its core function remains debated. By examining the function and regulation of sleep in the nematode *C. elegans*, we hope to uncover the phylogenic root of sleep. Across species, ligands of the Epidermal Growth Factor (EGF) family have well-characterized roles in development but also have a sleep-promoting effect when administered or overexpressed, the physiological significance of which is unknown. In *C. elegans* the sleep-inducing effect of EGF (LIN-3) overexpression is dependent on the activity of the sole EGF receptor homolog, LET-23, in a single interneuron called ALA. Here we show a physiological role of EGF-induced sleep in *C. elegans*. In response to a variety of stressful conditions, *C. elegans* adults engage in sleep-like behavior that is dependent on components of EGF signaling and the ALA neuron. Whereas wild-type animals are inhibited for feeding and locomotion for up to an hour after exposure to heat, cold, hypertonicity, and pore-forming toxin, animals that are mutant for components of EGF-mediated sleep resume feeding and locomotor activity significantly earlier. Importantly, we find that in response to extreme heat, sleepless ALA-defective animals are impaired for survival, demonstrating the benefit of stress-induced sleep. As pro-EGF transmembrane proteins must undergo proteolysis for release of soluble ligand, we sought to identify the sheddase(s) necessary for stress-induced sleep. In an RNAi screen for defective stress-induced sleep behavior we identified the *adm-4* metalloprotease. *adm-4* loss-of-function mutants are defective in stress-induced sleep and are also impaired for survival after extreme heat exposure. The function of ADM-4 in stress-induced EGF shedding appears to be conserved, as the mammalian ortholog ADAM17/TACE mediates stress-induced shedding of EGF domains in cell culture. We hypothesize that the sleep behavioral state evolved in order to divert resources toward recovery from cellular stress. The cellular homeostasis hypothesis for sleep is supported by cellular perturbations that accompany extended wakefulness, but such perturbations have not been shown to drive sleep behavior. Our work indicates that cellular stress can indeed induce sleep behavior, that this behavior is dependent on EGF signaling and the stress-responsive protease activity of ADM-4, and that this behavior is beneficial. As vertebrate EGF family ligands have been shown to undergo stress-induced shedding and act as somnogens, we posit that EGF signaling is part of a deeply conserved mechanism that contributes to sleep drive in response to cellular stress.

Disclosures: A.J. Hill: None. **J.M.N.G. Lopez:** None. **R. Mansfield:** None. **C. Van Buskirk:** None.

Nanosymposium

392. Brain Wellness: Metabolism and Energetics

Location: 206

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 392.01

Topic: C.01. Brain Wellness

Title: Insulin resistance predicts glucose uptake in Alzheimer's disease converters and early Alzheimer's disease patients

Authors: *A. A. WILLETTE, D. KAPOGIANNIS;
Natl. Inst. On Aging, Baltimore, MD

Abstract: Reduced glucose uptake in certain brain regions is a hallmark of Alzheimer's disease (AD). Peripheral insulin resistance (IR) in cognitively normal (CN) adults is associated with worse cognitive performance and reduced glucose uptake in these regions, as indexed by F18-fluorodeoxyglucose positron emission tomography ([F18]FDG). These relationships have not been examined across the AD spectrum, or in persons with Mild Cognitive Impairment (MCI) who either progressed to AD (MCI-P) or remained stable (MCI-S). We examined associations between peripheral IR, [F18]FDG uptake, and cognitive performance in CN, MCI, and AD as diagnosed at baseline, as well as in MCI-S and MCI-P as determined at 24 months after baseline. We studied 26 CN, 194 MCI (MCI-P n=39, MCI-S n=155), and 60 AD enrollees in the Alzheimer's Disease Neuroimaging Initiative (ADNI). Mean baseline [F18]FDG uptake was derived for five regions of interest (ROIs): lateral parietal cortex, posteromedial cortex, medial temporal lobe (MTL), hippocampus, and ventral prefrontal cortex (PFC). Two control ROIs, post-central gyrus and global cerebrum, were examined. Linear mixed modeling was used to assess baseline associations between the revised Homeostatic Model Assessment of Insulin Resistance (HOMA2-IR) index and mean [F18]FDG uptake in ROIs, as well as memory and executive function factor scores using mediation analysis. Among ROIs, glucose uptake was lower in a stepwise manner from CN, to MCI-S, to MCI-P, and to AD. For control regions, peripheral IR was not related to [F18]FDG uptake across or between diagnostic groups. For each of the five ROIs, there were significant HOMA2-IR * Diagnosis interactions. For instance, among AD subjects, HOMA2-IR predicted less [F18]FDG uptake in MTL (r=-0.37) and ventral PFC (r=-0.43). No associations were found for CN or MCI subjects. However, among MCI-P subjects, higher HOMA2-IR predicted more [F18]FDG uptake in hippocampus (r=0.59) and MTL (r=0.49) ROIs, whereas fit-lines were flat for MCI-S subjects. Among all 280 participants, higher MTL and PFC [F18]FDG uptake respectively predicted higher memory and executive function scores. HOMA2-IR was a partial mediator and significantly weakened associations between [F18]FDG uptake and cognitive performance. In conclusion, higher IR predicts hypermetabolism in MCI-P and hypometabolism in AD only among brain regions impacted by early AD. We speculate that higher IR may promote transient hypermetabolism in temporal and frontal ROIs in MCI converters, prior to eventual hypometabolism in AD. HOMA2-IR partially

mediates associations between temporal and frontal [F18]FDG uptake and cognitive performance.

Disclosures: **A.A. Willette:** None. **D. Kapogiannis:** None.

Nanosymposium

392. Brain Wellness: Metabolism and Energetics

Location: 206

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 392.02

Topic: C.01. Brain Wellness

Support: NIH Grant P50 AG016574

NIH Grant P50 NS072187

NIH Grant P01 AG003949

NIH Grant R01 NS080882

NIH Grant R01 NS065782

NIH Grant R01 AG026251

NIH Grant R01 NS076471

Title: Whole-genome sequencing to identify genes implicated in familial parkinsonian tauopathy

Authors: *M. Y. SANCHEZ-CONTRERAS[1], S. FUJIOKA[1], C. POTTIER[1], A. J. STRONGOSKY[2], B. F. BOEVE[3], J. E. PARISI[3], P. M. TACIK[1], N. AOKI[1], M. C. BAKER[1], V. SOSSI[4], D. W. DICKSON[1], A. STOESSL[5], O. A. ROSS[1], Z. K. WSZOLEK[2], R. RADEMAKERS[1];
[1]Neurosci., [2]Neurol., Mayo Clin., Jacksonville, FL; [3]Neurol., Mayo Clin., Rochester, MN; [4]Dept. of Physics and Astronomy, Univ. of British Columbia, Vancouver, BC, Canada; [5]Pacific Parkinson's Res. Centre, Div. of Neurol., Univ. of British Columbia & Vancouver Coastal Hlth., Vancouver, BC, Canada

Abstract: Tauopathies are a group of neurodegenerative disorders characterized by the pathological inclusions of phosphorylated tau protein. In some entities, as Alzheimer's disease, genetic variants have been proposed to contribute to tau pathology. However, genetic causes of

other less common tauopathies as progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) are poorly understood. To identify novel causal genes implicated in tauopathies, we provide a detailed study of a large family of 64 individuals with 8 affected patients with an inherited tauopathy that is likely autosomal dominant with reduced penetrance. The index case developed progressive speech and language difficulties at age of 64 years. Examination 4 years later disclosed non-fluent aphasia, word-finding difficulties, circumlocution, frontal release signs, and right-sided bradykinesia, rigidity, and pyramidal signs. She died 5 years after the symptomatic onset. Neuropathologic features included numerous ballooned neurofilament-positive neurons, tau-positive astrocytic plaques, and oligodendroglial coiled bodies, all typical of CBD. Two other family members were diagnosed clinically with parkinsonism and behavioral problems, 2 with Parkinson's disease, 1 with amyotrophic lateral sclerosis, 1 with dementia, and 1 with progressive gait and speech problem. DNA was available from the proband and one first-degree cousin, clinically diagnosed with dementia and parkinsonism at the age of 54. After exclusion of mutations in *MAPT, PGRN* and *LRRK2* and the expanded repeat in *C9ORF72* in these affecteds, we performed whole-genome sequencing. Analysis of the genomes of these patients filtered against variants observed in control individuals resulted in a list of novel and rare variants that were shared among the affecteds and follow an autosomal dominant inheritance pattern. This list of potential tauopathy variants was confirmed by Sanger sequencing and subsequently screened in a series of >750 population controls which resulted in a total of 6 confirmed-variants which were absent or very rare in controls: *SCN10A*, *OPRK1*, *CAPRIN2*, *UBN1*, *NEURL4* and *CCDC9*. The presence of additional variants in these candidate tauopathy genes currently is being studied in a series of pathologically-confirmed CBD and PSP cases as well as in 3 additional tauopathy families. This approach provides a select list of potential tauopathy genes by whole-genome sequencing in a family with pathologically confirmed CBD that may help identify novel pathways involved in pathological tau aggregation.

Disclosures: **M.Y. Sanchez-Contreras:** None. **S. Fujioka:** None. **C. Pottier:** None. **A.J. Strongosky:** None. **B.F. Boeve:** None. **J.E. Parisi:** None. **P.M. Tacik:** None. **N. Aoki:** None. **M.C. Baker:** None. **V. Sossi:** None. **D.W. Dickson:** None. **A. Stoessl:** None. **O.A. Ross:** None. **Z.K. Wszolek:** None. **R. Rademakers:** None.

Nanosymposium

392. Brain Wellness: Metabolism and Energetics

Location: 206

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 392.03

Topic: C.01. Brain Wellness

Title: Roles of ketone bodies in neuronal energy metabolism and plasticity

Authors: *K. MAROSI, A. CHENG, R. WAN, S. CAMANDOLA, M. P. MATTSON;
NIH, Baltimore, MD

Abstract: Dietary energy restriction (ER) and exercise have been shown to enhance the functional capabilities of the brain by inducing molecular and structural changes in the neuronal circuits. During ER and exercise a range of intracellular pathways are activated that modify metabolism, plasticity and stress resistance of the neurons. Brain derived neurotrophic factor (BDNF) plays a prominent role in the mediation of adaptive stress response of neurons to energetic challenges. Evidence shows that aerobic exercise and intermittent fasting increase BDNF expression and signaling in several brain regions, although the underlying cellular and molecular mechanisms remain to be determined. It is likely that BDNF is induced by changes in the brain network activity and by signals from the periphery during the shift cellular energy substrate utilization from glucose to ketones that occurs during fasting and vigorous exercise during which beta hydroxybutyrate (BHB) supplies over 50% of the brain metabolic energy needs. The aim of this study is to determine how BHB regulates neuronal energy metabolism and to investigate its role in synaptic plasticity. Treatment of primary cortical neurons with BHB resulted in the enhanced expression of BDNF gene. Cellular bioenergetics was evaluated by measuring mitochondrial oxygen consumption and the glycolytic rate. Treatment with BHB resulted in a shift from glycolysis towards oxidative phosphorylation, while the cells maintained ATP levels. In addition, AMP-activated protein kinase (AMPK) is activated in neurons in response to BHB treatment. Besides metabolic changes, incubation of neurons with BHB resulted in enhancement in the level of PGC1alpha, a protein that plays a critical role in mitochondrial biogenesis. Therefore ketone bodies might bolster neuronal bioenergetics to support neuronal growth and synaptic plasticity. In summary, via their ability to regulate BDNF signaling and energy metabolism of neurons, ketone bodies produced during fasting, exercise and ketogenic diets may promote optimal brain health. Our data also suggest that ketosis could be beneficial in neurological disorders associated with disrupted glycolytic capacity such as Alzheimer disease.

Disclosures: K. Marosi: None. **A. Cheng:** None. **R. Wan:** None. **S. Camandola:** None. **M.P. Mattson:** None.

Nanosymposium

392. Brain Wellness: Metabolism and Energetics

Location: 206

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 392.04

Topic: C.01. Brain Wellness

Support: University of Fribourg Research Pool

Swiss League against Epilepsy

Title: Metabolic sensing circuits of the insular cortex

Authors: I. DE ARAUJO SALGADO, *C. M. LAMY;
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Abstract: The insular cortex (IC) plays an important role at the body-to-brain interface. Neuroimaging studies have confirmed that this cortical area responds to changes in body states. Alterations in the processing of interoceptive signals by IC lead to neuropsychiatric symptoms such as anxiety. Despite their important clinical implications, the pathways underlying these effects have been little explored. Because glucose is one of the primary signals informing the brain on peripheral metabolic states, we investigated whether IC detects changes in glucose concentration and what effect it has on anxiety-like responses. Injection of 2-deoxyglucose (2DG), to mimic cellular glucoprivation, decreased anxiety-like behaviors in mice. This metabolic challenge also increased c-fos immunoreactivity in IC, suggesting a link between IC metabolic-responsive cells and behavior. We further dissected the underlying cellular mechanisms with whole-cell electrophysiological recordings from acute cortical slices. Changes in extracellular glucose concentrations reversibly altered the membrane potential and excitability of a subset of IC neurons. This effect persisted in the presence of synaptic inhibitors, indicating a cell-autonomous response. Morphological and molecular characteristics of glucose-responsive neurons were determined after recordings. These results indicate that IC contains metabolic sensing neuronal circuits that may contribute to the interaction between body metabolism and behavior in health and disease.

Disclosures: I. De Araujo Salgado: None. **C.M. Lamy:** None.

Nanosymposium

392. Brain Wellness: Metabolism and Energetics

Location: 206

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 392.05

Topic: C.01. Brain Wellness

Support: Natural Sciences and Engineering Research Council of Canada

University of Ottawa

Title: Effects of CRHR1 blockade on fear and spatial memory in relationship to BDNF mRNA and protein levels in the amygdala and hippocampus post global cerebral ischemia in male rats

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Abstract: Corticotropin releasing hormone (CRH) activation in the basolateral nucleus of the amygdala (BLA) and the CA1 subfield of the hippocampus has been shown to influence memory consolidation. Moreover, impaired fear memory, and reduced amygdaloidal CRH neurons and CRH receptor 1 (CRHR1) CA1 expression, have been reported following cerebral ischemia. Exposure to intense stressors induce increases in brain derived neurotrophic factor (BDNF) and its receptor TrkB in the BLA while levels are decreased in the CA1 that are paralleled by changes in dendritic spine density, a process in part mediated by CRHR1 activation. Considering the proposed role of CRH and BDNF in regulation of brain plasticity and memory processes, the current study evaluated the effects of blockade of CRHR1 on ischemia-induced behavioral impairments and mRNA and protein levels of BDNF and TrkB in the hippocampus and amygdala. Forty-eight male Wistar rats (n=12 per group) were subjected to sham surgery or global cerebral ischemia using the four vessel occlusion (4VO) model. ICV injection of Antalarmin (2µg/2µl) or saline was administered 30 min prior to ischemia. Rats were then assessed for fear and spatial learning in a Y-Maze inhibitory avoidance task, and in the Barnes Maze, respectively. Thirty days post reperfusion, a histological analysis of viable cells was completed in the CA1 and BLA and RT-PCR and Western blots performed for BDNF, TrkB and synapsin determination. Antalarmin-treated ischemic rats showed improved spatial memory in the Barnes Maze test and enhanced memory of an aversive experience in the Y-Maze. Antalarmin also enhanced CA1 neuronal survival although had no impact on neuronal injury at the BLA. Global ischemia led to increased BDNF and TrkB mRNA and protein expression in the amygdala, while decreased levels were detected in the hippocampus. Our findings suggest a role for CRHR1 activation in regulation of ischemia-induced CA1 neuronal injury and cognitive impairments, but not in ischemia-induced changes in hippocampal and amygdaloidal BDNF and TrkB expression.

Disclosures: P. Barra De La Tremblaye: None. **M. Bonneville:** None. **S. Schock:** A. Employment/Salary (full or part-time):; Research Associate, Children's Hospital of Eastern Ontario. **C. Thompson:** A. Employment/Salary (full or part-time):; Scientist, Ottawa Hospital Research Institute. **A.M. Hakim:** A. Employment/Salary (full or part-time):; Director,

Neuroscience Research, Ottawa Hospital Research Institute. **H. Plamondon:** A. Employment/Salary (full or part-time):; Associate Professors, University of Ottawa. 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.; Natural Sciences and Engineering Research Council of Canada.

Nanosymposium

392. Brain Wellness: Metabolism and Energetics

Location: 206

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 392.06

Topic: C.01. Brain Wellness

Support: P01 GM 084077

H. L Snyder foundation

Title: Azaphilones: A novel class of tau aggregation inhibitors

Authors: *S. PARANJAPE[1], Y.-M. CHIANG[2,4], A. SOMOZA[5], C. C. WANG[2,3], B. R. OAKLEY[1], T. C. GAMBLIN[1];
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Abstract: Alzheimer's disease (AD) is the most common form of dementia, affecting millions of people every year. The treatments currently available in the field reduce the rate of progression of the disease, but do not stop or reverse disease progression. Therefore current research is aimed at targeting the underlying molecular mechanisms such as the insoluble intracellular aggregates formed by the microtubule-associated protein tau. Tau aggregation correlates with dementia and neurodegeneration and is viewed as a potential therapeutic target for AD. We have previously identified three secondary metabolites obtained from Aspergillus nidulans as tau aggregation inhibitors: 2,-dihydroxyemodin, asperthecin, and asperbenzaldehyde [Paranjape, S.R. et.al. (2014)]. Because asperbenzaldehyde is a precursor to a class of molecules called azaphilones, we tested 11 azaphilones to determine their tau assembly inhibition properties in vitro. All the compounds tested inhibited tau filament assembly to some extent. However, 4 of the 11 compounds had the unexpected property of disassembling preformed tau aggregates in a dose-

dependent fashion by reducing the total amount of tau polymerization through a decrease in filament length and numbers. The hexapeptide motifs 275VQIINK280 and 306VQIVYK311 within the microtubule binding repeat regions are essential for both the process of tau aggregation and microtubule binding. Therefore, tau aggregation inhibitors could interfere with tau's normal function of stabilizing microtubules (MTs). We found that the most potent compounds inhibited MT assembly in the presence of tau, indicating that the compounds may be interacting with the hexapeptide motifs. These compounds require further validation in cellular and animal models, but are nonetheless very promising lead compounds for tau aggregation inhibitors and represent a new class of compounds with tau aggregation inhibitor activity.
Reference: Paranjape SR et.al. 'Inhibition of Tau aggregation by three Aspergillus nidulans secondary metabolites: 2,-dihydroxyemodin, asperthecin, and asperbenzaldehyde.' Planta Med. 2014 Jan;80(1):77-85.

Disclosures: S. Paranjape: None. **Y. Chiang:** None. **A. Somoza:** None. **C.C. Wang:** None. **B.R. Oakley:** None. **T.C. Gamblin:** None.

Nanosymposium

392. Brain Wellness: Metabolism and Energetics

Location: 206

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 392.07

Topic: C.01. Brain Wellness

Support: NINDS 1R21NS079324-01A1

Title: Effect of endurance exercise on the brain of Polg mutator mice

Authors: *J. CLARK[1], A. SALEEM[2], R. WANG[3], Y. DAI[3], X. MA[2], A. SAFDAR[2], M. TARNOPOLSKY[2], D. K. SIMON[3];
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Abstract: Physical activity is inversely related to the risk of Parkinson's disease (PD) and Alzheimer's disease (AD), and aerobic exercise is beneficial to patients with PD and AD; improving motor symptoms in PD and slowing cognitive decline in PD and AD. Despite these positive clinical data, mechanistic insight into neuroprotection by exercise is lacking. Mitochondrial dysfunction is heavily implicated in the progression of both PD and AD. Levels of somatic mitochondrial DNA (mtDNA) point mutations and deletions increase with age, and to a

greater extent in substantia nigra neurons in PD. To determine if endurance exercise can be beneficial to the brain when high levels of mtDNA mutations are present, we used a transgenic mouse that expresses a proofreading deficient form of mtDNA polymerase gamma (Polg). These Polg "mutator" mice exhibit accelerated accumulation of somatic mtDNA mutations, leading to a premature aging phenotype. Forced exercise in these mice normalizes muscle mitochondrial function, dramatically protects against systemic abnormalities and significantly extends lifespan. Now, we have used the same forced exercise paradigm to examine the effect of endurance exercise on the Polg mutator mouse brain with particular reference to the cerebral cortex and nigro-striatal system. The effect of endurance exercise on Polg mouse brain was examined in an unbiased manner using metabolomics alongside a targeted analysis of neurotrophic factors and other candidate mediators of exercise-associated neuroprotection. Metabolomics revealed important neurotransmitter abnormalities in Polg mouse brain that were normalized with exercise. Expression analysis revealed an altered pattern of neurotrophic factor expression in Polg mouse brain that was significantly impacted by exercise. Overall, the results presented here demonstrate that the Polg mouse model is a valuable resource for modelling deficits in the aging brain and reveal potential mechanisms that may contribute to the neuroprotective effects of exercise.

Disclosures: **J. Clark:** None. **A. Saleem:** None. **R. Wang:** None. **Y. Dai:** None. **X. Ma:** None. **A. Safdar:** None. **M. Tarnopolsky:** A. Employment/Salary (full or part-time):; Life Science Nutrition. 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.; Genzyme. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Genzyme - speaker honoraria, Prevention Genetics - speaker honoraria. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Life Science Nutrition and Exerkine Corporation.. **D.K. Simon:** None.

Nanosymposium

392. Brain Wellness: Metabolism and Energetics

Location: 206

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 392.08

Topic: C.01. Brain Wellness

Support: William E. Mabie snd Grace S. Mabie Fund

Title: Medial parietal cortex dysfunction as a biomarker of Alzheimer's disease and small vessel disease

Authors: *R. S. MILETICH[1], D. S. WACK[1], B. AJTAI[2], M. HOURIHANE[2];
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Abstract: Identifying biomarkers for neurodegenerative disease can provide useful clinical information for differential diagnosis and prognostication. Investigations with a number of potential Alzheimer's disease (ALZ) biomarkers are ongoing. However, the current nescience of the ALZ pathophysiologic process imposes serious limitations to biomarker discovery. A consistent finding with functional neuroimaging in ALZ has been temporal and parietal association cortex hypofunction, especially in the medial parietal lobes (MPL). We examined over 6,000 brain perfusion bicisate SPECTs from the CPET database at University at Buffalo. We discovered three general patterns of medial parietal cortex dysfunction in patients studied for memory loss, mild cognitive impairment or frank dementia. The first showed significant decrements of function in all 3 components of the medial parietal lobe: Precuneus (PREC), posterior cingulate cortex (PCC) and cortex of the medial parietal occipital sulcus zone (MPO). This involvement could be equivalent in all 3 zones, but often PREC was more severely affected. This pattern was usually present with other clinical and imaging stigmata supportive of the diagnosis of ALZ. The second pattern showed significant decreased function in the PREC with lesser involvement or no involvement of the PCC and MPO. This pattern was associated with clinical and imaging stigmata of cerebrovascular disease, particularly small vessel disease (SVD). The third pattern showed little or no involvement of any zone in the MPL. If any MPL hypofunction was present, it was in the PREC. This pattern was mainly associated with clinical and imaging stigmata of other neurodegenerative diseases, in particular frontotemporal lobar degeneration, or psychiatric disorders, such as affective illness. These findings support the use of basal physiologic functional imaging of the blood flow or metabolism of the MPL as a useful biomarker in the differential diagnosis of patients with cognitive complaints. Further, given the pattern overlaps, these findings implicate involvement of SVD in many cases of ALZ, particularly late onset ALZ (LOAD). These decrements of brain wellness in the MPL lend support to a multifactorial model of ALZ causation.

Disclosures: R.S. Miletich: None. **D.S. Wack:** None. **B. Ajtai:** None. **M. Hourihane:** None.

Nanosymposium

392. Brain Wellness: Metabolism and Energetics

Location: 206

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 392.09

Topic: C.01. Brain Wellness

Title: Intermittent energy restriction ameliorates cognitive impairment caused by a presenilin 1 mutation

Authors: *C. MAHARANA[1], H. CELIK[2], D. CAMERON[2], K. FISHBEIN[2], B. WUSTMAN[3], A. STEVENS[3], M. P. MATTSON[1,4];
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Abstract: Missense mutations in presenilin 1 (PS1) are the most common cause of early onset familial Alzheimer's disease (EOFAD) inherited in an autosomal dominant manner. PS1, a transmembrane protein, is the catalytic component of the gamma-secretase complex, but PS1 may also have gamma-secretase-independent functions. AD patients, including those with a PS1 mutation, exhibit deficits in brain cell energy metabolism that occurs relatively early in the disease process. We asked whether manipulations of dietary energy intake would modify cognitive function in PS1 mutant (M146V) knockin (PS1mutKI) mice, and whether PS1 mutations affect mitochondrial function. Wild type (WT) and PS1mutKI adult male mice (C57BL/6) were divided into different diet groups: control (C), intermittent fasting (IF) or high calorie diet (HCD). After 3 months of dietary intervention, mice were tested in the water maze. While PS1mutKI mice on the control diet were able to learn the location of the hidden platform during the acquisition trials, they exhibited impaired retention of the memory of the platform location in the probe trial three days after training. Maintenance on the IF diet ameliorated the memory retention deficit in the PS1mutKI mice, and the HCD worsened memory retention in both WT and PS1mutKI mice. To elucidate the impact of PS1 mutations on cellular bioenergetics we used a cell-based model with a human neuroglioma cell line engineered to express in an inducible manner WT or AD-causing mutant forms of PS1 that includes a range of ages of disease onset. We observed that PS1 mutations caused a reduction in mitochondrial membrane potential, increased vulnerability to glucose deprivation-induced degeneration, and an altered cellular respiratory capacity measured using an extracellular flux analyzer (Seahorse). We are currently performing magnetic resonance imaging spectroscopy analyses in WT and PS1mutKI mice in the C, IF and HCD groups to determine whether the PS1 mutation and diets modify levels of hippocampal metabolites and neurotransmitters. Our findings suggest that cognitive impairment caused by a PS1 mutation can be ameliorated by a simple dietary intervention that improves neuronal bioenergetics, suggesting a potential application to EOFAD patients.

Disclosures: **C. Maharana:** None. **H. Celik:** None. **D. Cameron:** None. **K. Fishbein:** None. **B. Wustman:** None. **A. Stevens:** None. **M.P. Mattson:** None.

Nanosymposium

392. Brain Wellness: Metabolism and Energetics

Location: 206

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 392.10

Topic: C.01. Brain Wellness

Support: NIH Grant R37NS34467

NIH Grant R37AG23084

NIH Grant RO1AG039452

Cure for Alzheimer Fund to BVZ

NIH Grant RO1AG035355

NIH Grant RO1AG027924

ACS Grant RSG-13-379-01-LIB

Title: PICALM regulates amyloid-b blood-brain barrier transcytosis and clearance

Authors: ***Z. ZHAO**[1], Q. MA[1], A. P. SAGARE[1], K. KISLER[1], E. A. WINKLER[1], A. RAMANATHAN[1], T. KANEKIYO[2], G. BU[2], N. C. OWENS[1], S. V. REGE[1], D. ZHU[3], M. MAEDA[4], T. MAEDA[4], B. V. ZLOKOVIC[1];
[1]Physiol. & Biophysics, USC, LOS ANGELES, CA; [2]Dept. of Neurosci., Mayo Clin., Jacksonville, FL; [3]Dept. of Chemical, Biol. and Bio-Engineering, North Carolina Agr. and Tech. State Univ., Greensboro, NC; [4]Brigham and Women's Hosp., Harvard Med. Sch., Boston, MD

Abstract: PICALM, the gene encoding phosphatidylinositol binding clathrin assembly protein, plays a key role in endocytosis, a process which regulates the function and internalization of cell receptors. PICALM was identified by genome wide association studies as a significant risk factor for Alzheimer's disease (AD), a neurodegenerative disorder characterized by neurovascular dysfunction, elevated amyloid β-peptide (Aβ), tau pathology and neuronal loss. The role of PICALM in disease pathogenesis remains, however, elusive. Here, we show that PICALM plays a central role in the molecular mechanisms regulating Aβ transcytosis and clearance across the

blood-brain barrier (BBB). Utilizing human brain endothelial cells and an in vitro monolayer model of the BBB, we show that Aβ binding to the low density lipoprotein receptor related protein-1 (LRP1), a key Aβ clearance receptor, recruits PICALM that binds to the YXXL motif within the cytoplasmic tail of LRP1 and initiates PICALM/clathrin-dependent endocytosis of Aβ-LRP1 complexes. We next show that PICALM directs Aβ traffic to different small Rab GTPases associated with the function of early endosomes and transcytosis and exocytosis of ligands, respectively, leading to Ab transcytosis. In mice, we show that Picalm deficiency diminishes Ab clearance across the BBB in vivo. Our data demonstrate that PICALM controls the clearance pathway of Aβ from brain across the BBB and, therefore, could be an important new therapeutic target for Aβ clearance therapy.

Disclosures: Z. Zhao: None. **Q. Ma:** None. **A.P. Sagare:** None. **K. Kisler:** None. **E.A. Winkler:** None. **A. Ramanathan:** None. **T. Kanekiyo:** None. **G. Bu:** None. **N.C. Owens:** None. **S.V. Rege:** None. **D. Zhu:** None. **M. Maeda:** None. **T. Maeda:** None. **B.V. Zlokovic:** None.

Nanosymposium

392. Brain Wellness: Metabolism and Energetics

Location: 206

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 392.11

Topic: C.01. Brain Wellness

Support: NWO NDNS+ Grant 'Desynchronization of Parkinsonian oscillations in the Subthalamic Nucleus'

Netherlands Brain Bank

Title: Gap junctions as modulators of synchrony in Parkinson's disease

Authors: *B. C. SCHWAB[1], H. G. E. MEIJER[1], R. J. A. VAN WEZEL[2], S. A. VAN GILS[1]; [1]Univ. of Twente, Enschede, Netherlands; [2]Radboud Univ., Nijmegen, Netherlands

Abstract: Parkinson's disease (PD) patients show abnormal levels of synchrony and low-frequency oscillations in the basal ganglia and the motor cortex. This altered neural activity is often associated with the motor symptoms of PD, but the mechanisms for the emergence of synchrony and oscillations remain debated. We suggest that neural gap junctions in cortex and basal ganglia contribute to this transition in activity. While gap junctions between interneurons

of cortex and striatum are well described, we do not know whether they appear in GPe and internal globus pallidus (GPi). Using confocal microscopy, we were able to detect the gap junction protein Cx36 in the human GPe and GPi, which was up-regulated in PD patients. Also the corresponding rat tissue showed Cx36 expression. Dopamine has already been described to modulate the conductance of gap junctions [1], especially also in the rat striatum, where dye coupling was increased after dopamine depleting 6-OHDA lesions [2]. In a conductance-based network model of the basal ganglia, we investigate the effect of gap junctional coupling in GPe and GPi on synchrony. While chemical synapses normally desynchronize the network, gap junctional coupling of sufficient strength is able to synchronize the whole basal ganglia. Also synchronized input from cortex to subthalamic nucleus has impact on synchronization, in particular in the case of numerous gap junctions in GPe. To describe the effect of gap junctional coupling between cortical interneurons on synchronized oscillations in the cortex, we introduce a diffusion term in a mean-field model. For high gap junctional coupling, large-amplitude oscillations of low frequency occur which are absent for low gap junctional coupling. Via the hyperdirect pathway, these oscillations could further synchronize the basal ganglia. We conclude that gap junctions can be a powerful trigger of synchrony in the basal ganglia. Their dependence on dopamine could explain the shifts of synchrony in PD. References 1. Li, H, Zhang, Z, Blackburn, MR, Wang, SW, Ribelayga, CP and O'Brien, J Adenosine and dopamine receptors coregulate photoreceptor coupling via gap junction phosphorylation in mouse retina. (2013) The Journal of Neuroscience, 33(7), 3135-3150. 2. Onn, SP and Grace, AA: Alterations in electrophysiological activity and dye coupling of striatal spiny and aspiny neurons in dopamine-denervated rat striatum recorded in vivo. (1999) Synapse, 33(1):1- 15.

Disclosures: B.C. Schwab: None. **H.G.E. Meijer:** None. **R.J.A. van Wezel:** None. **S.A. van Gils:** None.

Nanosymposium

392. Brain Wellness: Metabolism and Energetics

Location: 206

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 392.12

Topic: C.01. Brain Wellness

Support: AG000944

AG000928

Title: Metabolism analysis of young and aged Parkinson's disease-related α-synuclein transgenic mice

Authors: *X. CHEN, C. XIE, J. DING, L. SUN, H. CAI;
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Abstract: Parkinson's disease (PD), the most common degenerative movement disorder, is characterized by a progressive loss of dopaminergic neurons in the substantial nigra pars compacta and the formation of lewy bodies in the brain. A number of genetic factors have been identified in the etiology of PD. However, the mechanisms underlying neuron degeneration remain poorly understood. Metabolites are the best indicators of cell status, because they follow rapid fluxes and are extremely sensitive to the cellular changes. Therefore, tracking global metabolite alterations before and during neuron lost may provide mechanistic insights into the pathogenesis of the disease. In this study we used PD-related Pitx3-tTA::tetO-SNCA A53T (A53T) transgenic mice to determine the changes of metabolic profile in the brain. This transgenic mouse model overexpresses the PD-related α-synulcein A53T missense mutation in the midbrain dopaminergic neurons. The A53T mice showed severe motor disabilities and substantial neurodegeneration in the midbrain, which largely recapitulates the main neuropathological phenotypes of the PD patients. To perform the metabolism analysis, we collected brain tissues (forebrain plus midbrain) from four mouse groups with two different genotypes (A53T vs. non transgenic (nTg)) and two different ages (3-month-old vs. 18-month-old). Totally 32 samples were sent to Metabolon (Durham, NC) for metabolite profiling. After gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) analysis, 290 metabolites were identified in all samples. The two-way ANOVA test showed that in the subgroup comparison of 18-month-old nTg and 3 -month-old nTg, 10 metabolites showed statistically significant difference. While in the subgroup comparison of 18-month-old A53T and 3 -month-old A53T, 24 metabolites were significantly changed. We then used MetaAnalyst 2.0 to identify which pathways are contributed by these 10 and 24 metabolites separately. We found that steroid biosynthesis pathway is particularly affected by genotype, i.e., steroid biosynthesis pathway is only shown up in the list generated by 24 metabolites from A53T mice. Our results suggest that overexpression of PD-related α-synulcein A53T mutation may affect steroid biosynthesis during the progression of the disease. The implication of this finding is under investigation.

Disclosures: **X. Chen:** None. **C. Xie:** None. **J. Ding:** None. **L. Sun:** None. **H. Cai:** None.

Nanosymposium

392. Brain Wellness: Metabolism and Energetics

Location: 206

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 392.13

Topic: C.01. Brain Wellness

Support: Microbiology Foundation to LEB

NSF IGERT Grant DGE0965820 to RN

Title: Effects of high sucrose and high fat diets on memory, cognitive flexibility and gut microbiota

Authors: *K. R. MAGNUSSON[1], V. ELIAS[1], L. HAUCK[2], R. NATH[3], L. E. BERMUDEZ[2]; [1]Dept Biomed Sci, Coll Vet Med. & Linus Pauling Inst., [2]Dept Biomed Sci, Coll Vet Med., [3]Prog Human Dev & Family Sci., Oregon State Univ., CORVALLIS, OR

Abstract: The Western diet is high in fat and sucrose and has been shown to influence behavior and the gut microbiome. There is also growing evidence that altering the microbiome can influence the brain and behavior. This study was designed to determine whether diets high in fat or sucrose could influence anxiety, memory and/or cognitive flexibility and whether these changes were associated with specific changes in the gut microbiota. Two month old, male C57BL/6 mice were housed together as trios of siblings for two weeks on a control chow diet (13% kcal fat, 62% carbohydrate (CHO)). The mice were rotated between uncleansed cages until all the mice had been exposed similarly to establish a uniform microbiome baseline. Mice were then individually housed and, within each trio, were randomly assigned to high fat (42% fat, 43% CHO), high sucrose (12% fat, 70% CHO (primarily sucrose) or control chow diet groups. Fecal collections for microbiome analysis and step-down latency and novel object and novel location tasks were performed prior to the diet change and were repeated two weeks after the diet change. Water maze testing for long- and short-term memory and cognitive flexibility was conducted during weeks 3 and 4 post-diet change. There was no significant effect of diet on step-down, exploration or novel recognitions at 1- or 24-hour delay. The high sucrose group was significantly impaired in forming a spatial bias on day 1 for long-term memory and in the reversal trials, compared to control. There appeared to be an increase in perseveration in both the high sucrose and high fat groups during the reversal probe trials. The high sucrose group showed no evidence of short-term memory over a 10 minute delay. These results suggest that a high sucrose diet impaired long- and short-term memory and cognitive flexibility, including an increase in perseveration, suggesting effects on both hippocampus and frontal cortex. There were similar changes in the microbiomes of both the high sucrose and high fat diet groups in that both goups had increased percentages of the orders Clostridiales, Lactobacillales, and Erysipelotrichales and decreased levels of Bacteroidales. The microbiome analysis also showed differences that were unique to the high sucrose diet; Lactobacillales enterococcaceae was only detected in this group, and there was a greater increase of all Lactobacillales when compared to

the high fat. It is possible that the greater impact on behavior in the high sucrose group may be related to changes in Lactobacillales populations within the gut in combination with the other altered microbiome population ratios.

Disclosures: K.R. Magnusson: None. **V. Elias:** None. **L. Hauck:** None. **R. Nath:** None. **L.E. Bermudez:** None.

Nanosymposium

392. Brain Wellness: Metabolism and Energetics

Location: 206

Time: Monday, November 17, 2014, 1:00 PM - 4:30 PM

Presentation Number: 392.14

Topic: C.01. Brain Wellness

Support: This study was supported by the CHDI Foundation, Inc.

Title: Metabolism of 13C-labeled glucose and acetate reveals altered neuronal oxidation and glutamate-glutamine cycling in the zQ175 mouse model of Huntington's Disease

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[1]Yale Univ. Sch. of Med., New Haven, CT; [2]CHDI Foundation. Inc., Los Angeles, CA; [3]Yale Univ. Sch. Med., New Haven, CT

Abstract: Alterations in brain energy metabolism, including reduced glucose utilization and mitochondrial respiration, is observed in Huntington's Disease (HD) and HD animal models. Despite our detailed knowledge of the mutated gene and its poly-glutaminated (poly-Q) protein product, mutant huntingtin (mHtt), the mechanism underlying pathology and progression of HD remains enigmatic. There is an acute compelling need to apply new experimental tools to broaden the search for disease mechanism and identify potential therapeutic targets. Magnetic Resonance Spectroscopy (MRS) offers one such tool, which has been applied recently in HD afflicted individuals and HD animal models. MRS can be readily adapted to measure metabolic pathway flux by use of ^{13}C-labeled substrates In this study we measured the flow of ^{13}C label into glutamate, glutamine and GABA in 36 or 52 week old zQ175 het and non-carrier control mice following timed intravenous infusions of [1,6-$^{13}C_2$]glucose (neuronal and glial substrate) or [2-^{13}C]acetate (glial substrate) to determine whether dynamic turnover of the major amino acids linked to brain energy metabolism and neurotransmission (glutamate, GABA and glutamine) is altered in the zQ175 mice. To examine the dynamic turnover of the major amino acids linked to

brain energy metabolism and neurotransmission is altered in the zQ175 mice were infused with either [1,6-$^{13}C_2$]glucose (8 min) or [2-^{13}C]acetate (15 min) rapidly raising the respective concentrations and ^{13}C-enrichments to constant values. At the appropriate times, mice were quickly sedated (<30s) with isoflurane and euthanized by focused-beam microwave irradiation allowing brain tissue removal from cortex, striatum and thalamus without postmortem effects. Brain tissues were extracted using ethanol and the brain concentrations and ^{13}C enrichments of amino acids determined using ^{1}H-[^{13}C] MRS at 11.74T. We found that at ~52 weeks of age zQ175 het mice showed a significant reduction in ^{13}C fractional enrichment and ^{13}C concentration of glutamate, GABA, and glutamine in all three regions during infusion with [1,6-$^{13}C_2$]glucose. The percentage decreases in all regions were larger than in zQ175 het mice at 36 weeks. No difference was measured when acetate was infused with the exception of thalamus where ^{13}C fractional enrichments and ^{13}C concentrations of glutamine and glutamate were significantly lower, respectively. Estimation of metabolic rates from total ^{13}C labeling indicated reduced GABAergic and glutamatergic TCA cycles and reduced neurotransmitter cycling in the cortex. No reduction was found in the astroglial TCA cycle rate, similar to what was observed in ~36 week old zQ175 mice.

Disclosures: G.M.I. Chowdhury: None. **L. Park:** None. **O. Lavrova:** None. **G. Sanacora:** None. **D. Rothman:** None. **K.L. Behar:** None.

Nanosymposium

393. Novel Electrode Technologies

Location: 150A

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 393.01

Topic: G.04. Physiological Methods

Support: NINDS (1RC1NS068396-0110)

NIBIB (P41-EB002030)

U.S. Department of Energy (DE-SC0000957)

Title: Development of a high density and high channel count carbon fiber electrode array using a minimal and stackable silicon support structure

Authors: *P. R. PATEL[1], K. NA[2], H. ZHANG[3], T. D. Y. KOZAI[4], N. A. KOTOV[3], E. YOON[2], C. A. CHESTEK[1];

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Abstract: Ideal penetrating cortical neural electrodes are subcellular, bio-compatible, and have a high density & channel count. Many electrodes have been developed with all these goals in mind, but often sacrifice one property for another. Some of the smallest electrodes are manufactured using metals and MEMS processes which allows for a high density and high channel count array. These electrodes have a high Young's modulus, which aids in the insertion process. However, this high Young's modulus coupled with dimensions in the 10s of um, causes a lot of damage. Two alternatives to this approach are to create softer devices (e.g. lower Young's modulus) or make structures that are even smaller using more advanced fabrication techniques. Unfortunately, these methods often lead to the use of a shuttle, devices that are very large, or low channel counts. Our group has been actively exploring the use of carbon fibers with a minimal insulating layer of parylene-c that does not compromise the ultrasmall dimensions (d~7μm), which causes minimal scarring and improves recording performance. In addition, the inherently high Young's modulus helps during the insertion process. At lengths of up to 500μm we can insert 16 channel carbon fiber arrays, spaced at 76.2μm, with 100% success (n=5) and at lengths of 1mm we saw 86% success (n=5). Coupling this with a poly(ethylene-glycol) (PEG) coating has allowed us to insert longer fibers to a depth of 1.5mm and record chronic neuronal activity. To further facilitate insertion, speed up fabrication, and generate higher density arrays, a minimal silicon support structure was developed. The shanks (24μm(w) x 15μm(h)), each of which have a narrow groove in them, allow for faster assembly and can be stacked to generate a high channel count array. Each shank was separated by 150μm with shank lengths that varied from 250μm to 1mm. At these dimensions, it was determined that the shanks needed to be sharp, rather than flat, to insert with minimal dimpling. Longer length shanks were found to be better in securing each carbon fiber, ultimately allowing us to reach depths of 2mm, with the carbon fibers protruding 1mm beyond 1mm shanks. To further validate this design and the ability to record neuronal signals, a subset of silicon devices was fully functionalized with parylene-c and poly(3,4-ethylenedioxythiophene). Discernable actions potential were detected during an acute surgery on a Long Evans rat where the probe was implanted in the M1 region at a depth of 1mm. Neuroscience and neuroprosthetic applications will greatly benefit from this new platform that allows for high density recordings and reduced inflammation in a chronic setting.

Disclosures: P.R. Patel: None. **K. Na:** None. **H. Zhang:** None. **T.D.Y. Kozai:** None. **N.A. Kotov:** None. **E. Yoon:** None. **C.A. Chestek:** None.

Nanosymposium

393. Novel Electrode Technologies

Location: 150A

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 393.02

Topic: G.04. Physiological Methods

Support: NIH 1R01NS064318-01A1

IGERT 0903715

Title: Improved Utah Electrode Array long-term performance by identification and mitigation of failure modes

Authors: *R. B. CALDWELL[1], R. SHARMA[2], X. XIE[2], P. TATHIREDDY[2], F. SOLZBACHER[2], L. RIETH[2];
[1]Bioengineering, [2]Electrical Engin., Univ. of Utah, Salt Lake City, UT

Abstract: The silicon-micromachined Utah Electrode Array (UEA) is a well-established implantable neural interface device, but lifetime performance has been limited by electrode material degradation accelerated by electrical stimulation, physiological erosion of exposed silicon, and encapsulation failure from ingress of fluids and solutes from the extracellular space. We are conducting studies into innovative approaches that will mitigate these failure modes and drastically improve device performance lifetime. We hypothesize that 1: novel metallization and annealing strategies will prevent electrode degradation and stabilize single unit recordings by fully protecting underlying silicon from the physiological environment, and 2: a polymer-ceramic bilayer encapsulation will dramatically slow ingress and maintain chronic electrode impedances. Impedance measurements at 1 kHz have been performed at regular intervals on 48 of 96 UEA probes exposed to phosphate buffered saline at 57°C for 2 years; all probes incorporated a titanium-iridium oxide metallization scheme and our parylene-C - alumina bilayer encapsulation technology. Preliminary analysis showed a doubling of median impedance from an initial value of 100 kΩ over the course of soaking; SEM images illustrated gross metal degradation for measured channels, and electrode tip blunting across measured and unmeasured alike. These results strongly suggest that the mild voltage and current (10 mV, microamp range) associated with electrochemical measurements are sufficient to accelerate tip degradation, and saline-facilitated etching similar to that observed in prior studies of explanted chronic UEAs may be occurring at the electrode tips, which are vulnerable to physical damage. The lack of impedance reduction is attributed to well-functioning encapsulation, and SEM inspection showed little to no damage to the bilayer coating. Coating effectiveness has been demonstrated through leakage current measurements of interdigitated electrodes (IDEs) soaked in PBS at 67°C, which have shown that current through parylene-only encapsulated IDEs increases unacceptably past 1 nA within 2 months, while IDEs prepared with our bilayer coating maintain >40 pA leakage for

over 260 days, equivalent to over 5 years at 37°C. We will further confirm this performance by preparing UEAs with fully insulated electrode tips and measuring leakage current and impedance over time as they are exposed to a PBS bath at 57 and 67°C. Deinsulated UEAs incorporating our novel, proprietary metallization process will be also subject to PBS soaking and impedance spectroscopy measurements to validate electrode tip integrity over time.

Disclosures: **R.B. Caldwell:** None. **R. Sharma:** None. **X. Xie:** None. **P. Tathireddy:** None. **F. Solzbacher:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Blackrock Microsystems. **L. Rieth:** None.

Nanosymposium

393. Novel Electrode Technologies

Location: 150A

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 393.03

Topic: G.04. Physiological Methods

Support: NSF ECCS 1102067

Title: Integrating high resolution light sources onto Michigan implantable neural probes for optogenetic applications

Authors: ***F. WU**[1], E. STARK[3], M. IM[2,4], I.-J. CHO[2,5], E.-S. YOON[5], G. BUZSA´KI[3], K. D. WISE[2], E. YOON[2];
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Abstract: In order to advance the understanding of brain function and behaviors, it is critical to be able to monitor how neural circuits work together and perform computational processing. Because neural circuits are made of diverse cell types, selective activation/silencing of single neurons is required to identify the types of neurons being recorded by the sites and perturb the local circuits of cortical system in a controlled manner. Although optogenetics promises exciting new possibilities for the neuroscience community, to this date *there is still an unmet need for reliable implantable tools to precisely deliver light to the selected target neurons and simultaneously record from corresponding single neurons in a behaving animal*. Typically, light stimulation has been achieved by placing light sources on the surface of the brain or large fibers

in the brain parenchyma a few hundred microns from the recording sites. This approach inevitably activates many unmonitored neurons, making the separation between direct and population-mediated effects impossible. The high intensity used to activate deep neurons may generate multiple superimposed spike waveforms and considerable light artifacts. In the current application, we have designed, fabricated and tested a monolithically integrated optoelectronic probe. This novel approach provides spatially-confined stimulation of simultaneously monitored neurons by enabling local light delivery precisely above the recording sites at specific wavelengths of choice. The lithographically defined probe shank was designed with minimal dimensions to contain all necessary optical and electrical components, in order to minimize insertion-induced tissue damage and foreign body reactions, as well as to reduce alignment tolerance between the optical stimulation site and the electrical recording sites. We validated device feasibility by recording data from anesthetized animals. This technology can be easily expanded to any configurations delivering light to any specific locations and recording the photo-induced neural activity with electrode array. This class of devices will have a significant impact in capturing the full potential of optogenetics technology and accelerate our understanding of the role of specific neurons in behavior and complex circuits of the central nervous system such as neocortex and hippocampus. **Keywords**: optogenetics, neural probe
Theme and Topic: G.04.e. Electrophysiology: Electrode arrays **Presentation Preference (Complete)**: Nanosymposium (oral presentation)

Disclosures: F. Wu: None. **E. Yoon:** None. **K.D. Wise:** None. **I. Cho:** None. **E. Yoon:** None. **E. Stark:** None. **G. Buzsa´ki:** None. **M. Im:** None.

Nanosymposium

393. Novel Electrode Technologies

Location: 150A

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 393.04

Topic: G.04. Physiological Methods

Support: NIH R01NS062019

NIH 1K01NS066131

Title: *In vivo* 2 photon histology of novel electrode technologies directs next generation device design

Authors: *T. D. KOZAI[1], A. L. VAZQUEZ[2], X. CUI[1];
[1]Bioengineering, [2]Radiology, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Obtaining stable brain-activity readout with high-sensitivity is critical for many clinical applications as well as basic neuroscience research. Excitement over clinical possibilities for these technologies is curbed by concerns over long-term performance and health risks. Like other implants, a major challenge is the biological host response at the abiotic-biotic interface. To further investigate the dynamic in vivo histology to understand the benefits and limitations of novel electrode designs, in vivo multi-photon imaging was used to characterize the dynamic cellular response to implanted probes in mice. Microglial (CX3CR1-GFP), astroglial (sulfarhodamine101), neural vasculature (SR101/Texas Red/Quantum Dots) and neuronal activity (GCaMP3/Oregon Green Bapta-1) surrounding several novel electrodes were evaluated and compared to standard planar Michigan electrodes. These devices included L1CAM (neuronal a brain-derived neuronal specific cell adhesion molecule) coated Michigan electrodes, 8.5-micron diameter carbon fiber based Microthread composite electrode, Au nano-particle Layer-by-Layer flexible composite electrodes, lattice-style open architecture silicon electrode, Linear Blackrock array (with and without L1), microfluidic drug eluting probes, and glass microelectrode. Traditional electrodes showed substantial microglial activation, lamellipodia encapsulate sheath, increased neuronal deformation, and decreased neuronal activity compared to control tissue. Our findings suggest that nanometer-scale biomimetic L1CAM coating demonstrated the greatest ability to reduce glial activation acutely and chronically up to 3 months. Neither lamellipodia sheaths nor T-stage activation morphology were observed near the L1 coated region. Microglia surrounding the probes maintained ramified morphology. On the other hand, subcellular structured Microthread and lattice probes and improved flexibility minimally impacted acute glial activation. Subcellular featured electrodes did however, most dramatically impact neuronal health and neuronal activity. Lastly, dexamethasone eluting fluidic devices dramatically reduced glial encapsulation of the implant, but demonstrated global activation of nearby microglia. When combined with electrophysiolgical performance, electrochemical characteristics, and scanning electron microscopy data, these findings provide new insight for data driven, technology enabled device design.

Disclosures: T.D. Kozai: None. **X. Cui:** None. **A.L. Vazquez:** None.

Nanosymposium

393. Novel Electrode Technologies

Location: 150A

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 393.05

Topic: G.04. Physiological Methods

Support: NSF 078001

NIH7R43DA035545-02

Title: Precise modulation of *in vivo* neural network activities with focal release of 6,7-dinitroquinoxaline-2,3-dione (DNQX) directly from microelectrodes

Authors: *Z. DU[1,2], D. J. SIMONS[3,2], G.-Q. BI[4,2,5], X. T. CUI[1,2];
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Abstract: Pharmacological modulation of neural activities is an essential technique for the study of neural network functions and treatment of diseases. The fast and precise modulation of small neural network may treat neurological diseases such as epilepsy or Parkinson's disease. Traditional methods for locally delivering neural modulators such as cannular, microfluid device and iontophoresis suffer from certain drawbacks, including drug leakage, increased device size, complicity and fragility, and drug delivery channel clogging. On any neural recording or stimulation electrode surface, conducting polymers can be formed by electropolymerization with negatively charged molecules incorporated. When negative current is injected into the polymer film, these dopant molecules can be released from the composite film. In this study, a novel drug delivery system was demonstrated with DNQX (6,7-dinitroquinoxaline-2,3-dione) release in rat somatosensory (S1) barrel cortex. The spatial and temporal resolution of drug delivery is demonstrated with model molecule fluorescein with direct fluorescent microscopy measurements. An effective concentration of drug molecules can be released by single electrical trigger, within a zone of at least 200 μm that lasts more than 1.5 seconds. The amount of single trigger released drug decreases with the numbers of releases, but a stable amount can be released more than 50 times. The conductivity of the polymer film is disrupted by incorporating the DNQX molecules. To overcome this issue, a bilayer structure of polymer composite was developed. The bottome layer is a highly conductive PEDOT-fCNT (Poly(3,4-ethylenedioxythiophene)-functional carbon nanotubes) and the top layer consistes of DNQX loaded polypyrrole (Ppy) -fCNT which has higher release capacity. Barrel cortex (SI) is selected since the neural activity of layer IV neurons reliably represent sensory stimulation from facial whiskers in a topographical manner,. Multi-whisker air puff stimulation is utilized to elicit repeatable neural network activity patterns. Then this neural activity was immediately and locally suppressed by released DNQX. demonstrating high spatial and temporal precision of the novel drug delivery system. With the ease of being incorporated in any existing extracellular

neural electrode devices, this focal release technology may find use in a wide range of neuroscience studies and potentially therapeutic devices.

Disclosures: **Z. Du:** None. **X.T. Cui:** None. **D.J. Simons:** None. **G. Bi:** None.

Nanosymposium

393. Novel Electrode Technologies

Location: 150A

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 393.06

Topic: G.04. Physiological Methods

Title: Electrode development and characterization for chronic stimulation

Authors: ***V. TOLOSA**, K. SHAH, K. LEE, A. TOOKER, S. FELIX, S. PANNU; LAWRENCE LIVERMORE NATIONAL LAB, LIVERMORE, CA

Abstract: Neuromodulation through electrical stimulation is being investigated for a growing number of nervous system disorders. Subsequently, efforts are underway to develop a variety of neuromodulation therapies for humans. Despite this growth in applications and need, almost all current commercially available stimulation devices are hand-made (e.g., cochlear and DBS implants). Mass production through microfabrication would be cost-effective and enable miniaturization. One disadvantage of microfabrication is the inherent thin films of electrode materials. Inevitably, any effective stimulation parameter causes some material dissolution. Planar thin-film electrodes, though suitable for some applications, are not appropriate in cases where higher current densities are required. A number of research efforts are focused on improving the longevity of microfabricated electrodes for such applications, mainly through increasing the effective surface area of a film and/or exploiting charge injection capacities of different materials. Here we present the development of four different microfabricated platinum electrodes: thin-film platinum (TFPt), electroplated Pt (PtBlk), nano-clustered Pt (NCPt), bulk platinum (Bulk-Pt). Each material is fabricated differently; TFPt is sputtered at a wafer level, PtBlk is electrodeposited on individual TFPt seed electrodes, NCPt is deposited using a proprietary method at a wafer level, Bulk-Pt is mechanically attached to individual TFPt seed electrodes. Wafer-level deposition methods provide the benefit of mass production and reproducibility, but add complexity to the device fabrication. Electrode material deposition post-fabrication provides modularity and enables the incorporation of a bulk metal. TFPt is a standard, planar thin-film electrode used as a benchmark. PtBlk and NCPt offer high effective surface areas to reduce dissolution rates. Bulk-Pt offers a microfabricated bulk alternative to hand-made

bulk electrodes. All four materials were fabricated with the same geometric diameter. Each material was characterized and compared. The advantages and disadvantages of each material, suggest that there is not a one-size-fits-all policy when it comes to choosing the optimal material for an application. As new electrode materials continue to be developed, fabrication feasibility and application of the material must be considered when designing a neural interface. Understanding how the materials perform and degrade in relation to fabrication is equally important.

Disclosures: **V. Tolosa:** None. **K. Shah:** None. **K. Lee:** None. **A. Tooker:** None. **S. Felix:** None. **S. Pannu:** None.

Nanosymposium

393. Novel Electrode Technologies

Location: 150A

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 393.07

Topic: G.04. Physiological Methods

Support: NSF Grant 1152658

Title: Next generation, ultra-high density implantable nano-optoelectrical neural interfaces

Authors: ***M. CHAMANZAR**[1], M. BORISOV[2], D. J. DENMAN[3], M. M. MAHARBIZ[1], T. J. BLANCHE[3,2];
[1]Univ. of California Berkeley, Berkeley, CA; [2]E3 Neurotechnology, Seattle, WA; [3]Allen Inst. for Brain Sci., Seattle, WA

Abstract: We discuss the design and implementation of minimally invasive, ultra-high density optrodes for simultaneous high-resolution electrophysiology and optogenetic stimulation of subsets of neurons in multiple brain areas. The neural interface consists of multiple independent 64-channel implantable probes connected to a lightweight headstage (1.3 g for the 256 channel configuration, suitable for mice) through monolithically integrated compliant parylene cables (6 μm thick with a stiffness of 2.5x10-6 N/m). The probes can be independently implanted in multiple locations of the brain (Fig. 1a). The implantable part of each probe is a compact (50 μm × 20 μm × 2 mm) silicon shank with 64 double-sided recording sites. The recorded extracellular action potentials from the recording sites are routed through densely integrated 250 nm interconnects, fabricated using an optimized deep UV (DUV) lithography technique. 16 ultracompact (2 μm × 2 μm) polymer photonic waveguides embedded in parylene are integrated

on shank in a separate layer (Fig. 1c), providing local optogenetic stimulation of the neural population adjacent to the probe. We evaluated the coverage and effective spatial resolution by combining the optrodes with in vivo 2 photon imaging of GCamp6-expressing neurons in transgenic mice. The system is modular and can be expanded to interrogate thousands of neurons by adding to the headstage stack and 64-channel probes. The entire manufacturing process, including the nanofabrication of the optrodes, post-fabrication assembly, and surgical implantation procedures are designed to be scalable, low cost and high-yield. In this presentation, we will discuss the details of the design and manufacturing of the probes and procedures for high throughput, in-vivo multi-scale electrophysiology recording and optogenetic stimulation in awake, freely-behaving transgenic mice.

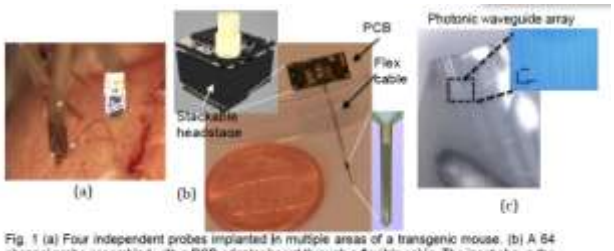

Fig. 1 (a) Four independent probes implanted in multiple areas of a transgenic mouse. (b) A 64 channel probe assembled with a PCB adaptor board through a flexible cable. The inset shows the stackable headstage module. (c) An array of photonic waveguides realized on a flexible substrate.

Disclosures: **M. Chamanzar:** None. **M. Borisov:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); E3 Neurotechnology. **D.J. Denman:** None. **M.M. Maharbiz:** None. **T.J. Blanche:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); E3 Neurotechnology.

Nanosymposium

393. Novel Electrode Technologies

Location: 150A

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 393.08

Topic: G.04. Physiological Methods

Support: NIH Grant 1R43NS079023-01A1

Title: Customizable and flexible polymer neural microelectrode array

Authors: *S. NEGI[1], A. HOGAN[3], S. BUTLER[3], X. XIE[2], R. BHANDARI[3];
[1]Electrical and Computer Engin., [2]Univ. of Utah, Salt Lake City, UT; [3]Blackrock Microsystems, Salt Lake City, UT

Abstract: In the last two decades though the field of neuroprosthetics has gained tremendous momentum through the development of novel architectures for neural interfaces, however, till date there is no device which can be nomenclature as the "gold standard" in the field. As a result the research and technology environment at each laboratory is unique and require a high-level of customization and integration. The objective of this research is to devise a **"one-in-all technology"** that encompasses the assets (such as ultra high aspect ratio, multi-channels, flexible substrate, interconnection etc.) of the silicon (such as the Utah and Michigan array) and polymer based arrays and mitigates their limitations. Most importantly it provides a highly customized and "poor man's solution" to research laboratories. We present a novel platform micro molding technique, which enables devolvement of highly customized 2D &3D probes as per the neuroscientists' needs and interests at an affordable budget without compromising on the quality and integrity of the device. The technology offers highly customized design rules which include surface and penetrating electrodes. It allow the end users for the first time to dictate their own electrode design (specified within the design rules) as per their hypothesis, and choose the number of active channels per shaft, location and material used for active sites, shape and size of the shaft etc. The microelectrode arrays are made of flexible material such as polyimide and parylene-C to mitigate the tissue insult. However, the neural electrodes have backbone of glass, which give the probes mechanical strength to withstand the insertion forces during penetration of the tissue. The active sites are from 15 to 30 um and are of platinum, iridium oxide or conducting polymer PEDOT (fig left). The probe length is from 1 cm to 0.5 cm having 8 to 64 channels. The active channels can be on both sides of the probes increasing the channel count to 128. None of the planar probes we are aware of have the capability to fabricate active sites on both sides of the probes. Also, the micro-holes can be made in the probe to mitigate foreign body response (fig right). The holes can be used in different ways: (1) reduce the mass of the probes hence reduce solid-tissue interface, (2) drugs can be stored in these holes before implantation which can slowly diffuse into the tissue and increase neuronal growth, and (3) constant drug or light delivery by making microchannels along the shaft of the probes. To summarize, we will demonstrate in the presentation about the versatility of the technology in making wide configuration of probes and in vivo testing in rats.

Disclosures: **S. Negi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Blackrock Microsystems. **A. Hogan:** None. **S. Butler:** None. **X. Xie:** None. **R. Bhandari:** None.

Nanosymposium

393. Novel Electrode Technologies

Location: 150A

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 393.09

Topic: G.04. Physiological Methods

Support: NYU Grand Challenge Award

Taking Flight Award from Citizens United for Research in Epilepsy

Title: A flexible, low-cost 60-channel μECoG array for use in rodents

Authors: M. TRUMPIS[1], M. INSANALLY[1], R. C. FROEMKE[1], *J. VIVENTI[2,1];
[1]New York Univ., New York, NY; [2]Electrical and Computer Engin., Polytechnic Inst. of New York Univ., Brooklyn, NY

Abstract: Micro-electrocorticography (μECoG) provides a large scale view of spatiotemporal dynamics across multiple cortical areas. To characterize sensory processing in auditory areas we have developed a low-cost, flexible μECoG array. The array was fabricated using commercial manufacturing at a cost of just $26 each. The electrode contacts were arranged in an 8×8 grid, with 60 recording sites (corners excluded). The gold electrode contacts were 203 μm in diameter with mean impedance of 32kΩ @ 1 kHz. The contacts were located at 406μm pitch, covering a recording area of 3.25x3.25 mm. In concert with the array, we developed a compact and low cost ($100) amplified and multiplexed headstage to enable chronic recordings with a small number of external wire connections (11). The noise floor of the headstage was 5.90 μV RMS in the 1-300 Hz band, yielding a physiological signal to noise ratio of 24.17 dB. We validated the quality of the array and recording system by mapping the frequency response area (FRA) of electrode sites in rat auditory cortex. The distribution of site FRAs were consistent with the tonotopic organization of auditory cortical areas. FRAs were mapped on 41 of 60 sites, and tone-evoked potentials exceeding 1 mV peak-to-peak were clearly visible on single trials. FRAs mapped with single-trial partitions of the dataset were highly consistent with FRAs mapped from trial-averaged data. The average of single-trial maps matched the FRA maps of trial-averaged data with Pearson correlation 0.94 +- 0.04 across sites (mean +- std). d' reliability for tone-responding sites was 2.52 +/- 0.83. The signal redundancy from site-to-site was assessed by the roll-off of Pearson correlation and magnitude-squared coherence (MSC) with distance. Correlation between sites decreased linearly with distance, falling to half at a distance of approximately 2 mm. MSC fell to half of its maximum at distances of 1.42 mm to ≤ 406 μm between 1-75 Hz. As a more practical measure of information captured in the array recording, we also report the ability of a

logistic regression classifier to discriminate tones based on neural response. The classifier was trained on an optimal linear projection of the 100 ms post-tone response of all tone-responsive sites. The classifier performance for the 7 tones supporting 90% of the frequency response mass was 0.78 +/- 0.06 at half-octave resolution. Our electrode was designed to utilize high quality flex PCB manufacturing to facilitate reproducibility and reduce cost. The recording system was designed for chronic implantation, allowing us to obtain high quality, dense μECoG recordings from behaving animals in the future, as in Insanally et al. and Carcea et al. SFN 2014.

Disclosures: **M. Trumpis:** None. **M. Insanally:** None. **R.C. Froemke:** None. **J. Viventi:** None.

Nanosymposium

393. Novel Electrode Technologies

Location: 150A

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 393.10

Topic: G.04. Physiological Methods

Title: Carbon nanotube fiber implantable neural electrodes for chronic recording and stimulation

Authors: ***F. VITALE**[1], S. R. SUMMERSON[2], B. AAZANG[2], C. T. KEMERE[2,3], M. PASQUALI[1];
[1]Chem. and Biomolecular Engin., [2]Electrical and Computer Engin., Rice Univ., Houston, TX;
[3]Dept. of Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Standard metal electrodes for neural recording and stimulation face several problems, from low charge injection limits to instability and delamination. Carbon nanotubes (CNT) have been used to fabricate microelectrodes for in vitro stimulation and for in vivo electrophysiology, however due to the challenge of translating the single molecule properties in microscopic assembly and the difficulties of reliably fabricating CNT electrodes the potential of CNT-composite microelectrodes has not been fully explored. Recently, we have produced carbon nanotube fibers (CNTf), which are highly conductive, electrochemically stable, strong, flexible, and microscale in size. These properties make it an attractive alternative to current materials use in microelectrode fabrication. We evaluate our CNTf for use as a recording electrode and stimulation electrode. In Long-Evans rats (N = 2), we implant four-channel electrodes (a.k.a. tetrodes) with three channels made from gold-plated nichrome (NiCr) wire and one channel made of CNTf filament. Neural activity is recorded while the rats behave spontaneously. The local field potential signal is recorded on all channels of the tetrodes at a sampling rate of 30

kHz. Additionally, threshold-crossing event waveforms from all channels are saved when activity on one channel exceeded a tetrode-specific threshold, which was set between 35 and 60 μA. Individual units are identified from the threshold-crossing events by clustering spikes using peak amplitude and spike width. The quality of the spiking activity recorded using the CNTf compared to the NiCr is similar, as quantified by the average peak-to-peak voltage of the spike waveforms and the signal-to-noise ratio of the high pass filtered signal. Additionally, the recording quality was stable over a period of two weeks of recording. The CNTf is also used to fabricate stereotrodes for stimulating neural structures. We evaluate the performance of a CNTf stereotrode as a deep brain stimulation (DBS) electrode in hemi-Parkinsonian Long-Evans rats (N = 4) using a rotation test, which is a standard test of motor asymmetry. We find that the electrode works just as well as standard commercial electrodes in terms of the therapeutic benefits achieved with GPi-DBS in the hemi-Parkinsonian rat model. Together with the recording results, we find that microelectrode fabrication with CNTf has great potential. Unlike standard metal recording electrodes, CNTf electrodes need no electroplating to achieve the desirable impedance spectrum, which means that the same implanted electrode may be used for obtaining high-quality neural recordings and microstimulation.

Disclosures: F. Vitale: None. **S.R. Summerson:** None. **B. Aazang:** None. **C.T. Kemere:** None. **M. Pasquali:** None.

Nanosymposium

393. Novel Electrode Technologies

Location: 150A

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 393.11

Topic: G.04. Physiological Methods

Support: ERC StG 259419

Title: Chronic spike recordings from a regenerating peripheral nerve in awake and freely moving rats

Authors: *S. P. LACOUR[1], K. MUSICK[1], J. RIGOSA[2], M. CAPOGROSSO[2], S. WURTH[2], S. MICERA[2];
[1]LSBI, [2]TNE, EPFL, Lausanne, Switzerland

Abstract: Reliably interfacing a nerve with an electrode array is one of the approaches to restore motor and sensory functions after nervous system injury or disease. Accomplishing this with

current technologies is challenging as the electrode-neuron interface often degrades over time, and in awake moving animals, surrounding myoelectric signals contaminate the neuro-signals. We have optimized the design, fabrication and implementation of a regenerative nerve electrode interface so that chronic electrical recordings from the regenerating sciatic nerve fibers are reliably collected from awake, behaving rats. This result has important implications for understanding functional nerve regeneration and developing novel ways of driving prosthetics that interface directly to nerves stumps on amputee patients. At the start of the experiment, the left sciatic nerve of four male Lewis rats was sectioned, and each stump was sutured into a custom-made regenerative conduit. The conduit is made of polydimethyl-siloxane (PDMS) and consists of a 7 x 10 array of stacked microchannels. Each channel has a rectangular cross-section of 110 x 120 μm2, and the length of this conduit (the regeneration gap) is 4 mm. A microchannel electrode configuration is used as a means of locally amplifying the axonal signal. When an action potential travels along the length of an axon, a very small electrical current is produced in the extracellular space. By confining this current within a small volume, the corresponding potentiall is amplified compared to the same signal within a bulk volume. This allows us to record neuronal spiking within the very noisy environment of an awake, moving animal. Within ten of these microchannels, there is an electrode at the midpoint of the channel. Over a period of 10 weeks, the nerve regenerated through this array of microchannels and the electrodes were used to obtain electrical signals from within the nerve. The electrodes were connected to wires that run subcutaneously to a headstage to allow frequent and non-invasive recordings from the nerve. Once per week starting at 8 days after surgery, the rats completed a task which involved walking the length of a runway. During the task, gait kinematics and neuronal signals were recorded. By analyzing the spike rates over time, we can track the functional regeneration of the nerve in a real-time fashion. We can also use the results from this experiment towards the development of a smart prosthetic to interface directly with the peripheral nervous system. Using the correlation of firing rates with leg movements in a predictive fashion would provide a means of driving this prosthetic in a more natural manner.

Disclosures: **S.P. Lacour:** None. **K. Musick:** None. **S. Micera:** None. **J. Rigosa:** None. **M. Capogrosso:** None. **S. Wurth:** None.

Nanosymposium

393. Novel Electrode Technologies

Location: 150A

Time: Monday, November 17, 2014, 1:00 PM - 4:00 PM

Presentation Number: 393.12

Topic: G.04. Physiological Methods

Support: NSF (CBET-1253890)

Center for Materials Science and Engineering (DMR-0819762)

Center for Sensorimotor Neural Engineering (ERC)

Supported by a grant from the Simons Foundation to the Simons Center for the Social Brain at MIT

McGovern Institute for Brain Research

Title: Flexible and multimodal fiber probes for chronic optical, electrical and chemical interrogation of brain circuits

Authors: *U. P. FRORIEP[1,2], A. CANALES[3], X. JIA[1], C. LU[3], R. A. KOPPES[1], C. TRINGIDES[3], J. SELVIDGE[3], Y. FINK[1,3], P. ANIKEEVA[3,1];
[1]Res. Lab. of Electronics, [2]Simons Ctr. for the Social Brain, [3]Dept. of Materials Sci. and Engin., MIT, Cambridge, MA

Abstract: Neural probes are essential for the mapping of neural circuits and neuroprosthetic brain machine interfaces. However, their chronic applicability is currently limited due to deterioration of the conductive interfaces, inflammatory tissue response, and a successive decrease in signal to noise ratio. It has been hypothesized that these observations result from a mismatch in elastic moduli of neural probes in comparison to neural tissue. Furthermore, systems neuroscience experiments often make it necessary to combine neural recording with optogenetic stimulation and pharmacological intervention, which ideally would be performed within a single, biocompatible, flexible, and multifunctional device. Here we present a new type of neural probes, consisting of biocompatible polymers or polymer-metal composites, fabricated by using a fiber drawing process. This method allows the design of the probes' layout on the macroscale using conventional machining, followed by size reduction of up to 200 times with preserved cross-sectional geometry. Multiple drawing steps can further reduce the device dimensions, enabling micro- to nanoscale resolution. By employing this process we developed several types of flexible neural probes (bending stiffness between 4 and 149 N/m) including multielectrode arrays and multifunctional devices which seamlessly integrate recording, optogenetic stimulation (transmission loss 1.6-2.7 dB/cm), and fluidic delivery capabilities. We confirmed the capabilities of our devices under acute and chronic freely-moving conditions in wildtype and transgenic mice in vivo. We successfully recorded both endogenous and stimulated single and multi unit activity with signal to noise ratio of up to 20, and injecting bicuculline methiodide through the device into the brain resulted in a significant change in the neural activity profile. Thus, our flexible probes facilitate chronic recording and intervention in the central and peripheral nervous system.

Disclosures: **U.P. Froriep:** None. **A. Canales:** None. **X. Jia:** None. **C. Lu:** None. **R.A. Koppes:** None. **C. Tringides:** None. **J. Selvidge:** None. **Y. Fink:** None. **P. Anikeeva:** None.

Nanosymposium

481. Oscillations and Synchrony

Location: 152B

Time: Tuesday, November 18, 2014, 8:00 AM - 10:45 AM

Presentation Number: 481.01

Topic: B.09. Network Interactions

Support: NIH R21 MH093858

NIH R01 MH102471

Title: Neural activity underlying functional connectivity MRI

Authors: ***J. LI**[1], W. BENTLEY[2], L. SNYDER[2];
[2]Dept. of Anat. & Neurobio., [1]Washington Univ. In St Louis, St Louis, MO

Abstract: Analyses of inter-regional correlation of fluctuations in the blood oxygen level dependent (BOLD) fMRI signal, otherwise known as functional connectivity or fcMRI, have provided enormous insight into the functional architecture of the brain. Functional connectivity has been used to describe the topology of functional networks and how these networks change over time with development, training and pathology. However, it is largely unknown how neural activity corresponds to BOLD correlation. We addressed this issue by comparing oxygen correlation with simultaneously recorded neural activity. Adjacent electrodes (0.5 mm spacing) were used to simultaneously record local oxygen level (oxygen polarography, similar to BOLD: Bentley et al 2013, SFN abstract) and neural activity, respectively, from two nodes in the default mode network (left and right posterior cingulate cortex) and two nodes in the visual/attention network (left and right V3). With this method, we found that slow (0.01-0.1Hz) oxygen fluctuations show long-distance correlation within a network, similar to fcMRI (Bentley et al. 2014, SFN abstract). Here we show that slow (0.01-0.1 Hz) fluctuations of both local field potential (sLFP) and multi-unit activity (sMUA) are also highly correlated between regions within a network. Further we demonstrate that sLFP and sMUA share information with the local oxygen signals. Regressing out shared sLFP and sMUA information from oxygen signals, respectively, reduces in-network oxygen correlation by 45 and 60%. Regressing out both signals reduces correlation by 70%, an amount significantly greater than that obtained from either regression alone. This suggests that oxygen fluctuation reflects both local dendritic currents and

action potentials, and long-distance oxygen correlations are produced in response to long-distance correlations in neural activity.

Disclosures: J. Li: None. **W. Bentley:** None. **L. Snyder:** None.

Nanosymposium

481. Oscillations and Synchrony

Location: 152B

Time: Tuesday, November 18, 2014, 8:00 AM - 10:45 AM

Presentation Number: 481.02

Topic: B.09. Network Interactions

Support: CIHR Grant MOP-102599

Human Frontier Science Program (RGY0080/2008)

Title: Laminar-specific phase amplitude coupling correlates with spontaneous hemodynamic fluctuations, but is a lesser predictor than band limited power of local field potentials

Authors: *R. C. SOTERO, A. BORTEL, S. NAAMAN, A. SHMUEL;
McGill Univ., Montreal, QC, Canada

Abstract: Resting state fMRI signals correlate more consistently with locally measured fluctuations in power of gamma band of local field potentials (LFP) [1,2] than lower-frequency bands [2]. However, resting-state functional-connectivity (RSFC), as measured by fMRI, is mediated by inter-areal synchronization of low-frequency LFP [3]. It was proposed that RSFC is mediated by inter-areal synchronization of low-frequency LFP and by local phase-amplitude coupling (PAC) between low frequency bands and the gamma band [3]. If PAC is the mechanism linking fMRI based- and neurophysiology-based functional connectivity, it should be correlated with local hemodynamic signals. To test this hypothesis, we simultaneously recorded spontaneous LFP using laminar probes and optical imaging signals (OIS) in rat area S1. Spontaneous OIS blood-oxygenation was calculated by averaging pixels from the cortical gray matter within 1mm radius of the probe. The band limited power (BLP) was computed by integrating the LFP spectra within each frequency band of interest: delta, theta, alpha, beta, low gamma (30-50 Hz), middle gamma (50-100 Hz) and fast gamma (100-150 Hz). In addition, we computed PAC for all possible combinations of phases and amplitudes. Linear and nonlinear correlation between BLP and OIS, and linear correlation between PAC and OIS were then computed. We calculated the coefficient of determination, with higher values indicating which

model and which frequency band explains the OIS best. Our results (Figure 1) show that while the three gamma bands are not individually a better predictor of OIS than lower frequency bands, when taken as a whole, the gamma band is the best predictor of OIS. Moreover, our results highlight the importance of nonlinear mechanisms in the generation of hemodynamic signals. PAC, a nonlinear phenomenon, is a better predictor of OIS than linear models of BLP/OIS, although worse than nonlinear models of BLP/OIS. [1] Shmuel, A., Leopold, D. A. (2008). Hum Brain Mapp 29, 751-761. [2] Schölvinck et al. (2010).PNAS 107, 10238-10243. [3] Wang et al. (2012). Neuron 76, 1010-1020.

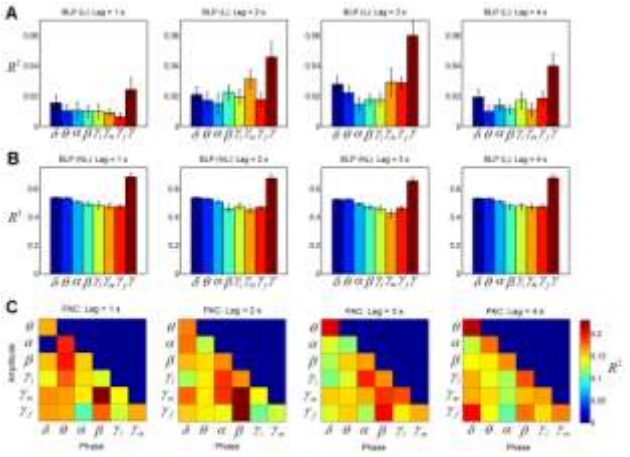

Figure 1. Coefficient of Determination. A) linear correlation model between BLP and OIS. B) nonlinear correlation model between BLP and OIS. C) linear correlation model between PAC and OIS.

Disclosures: **R.C. Sotero:** None. **A. Bortel:** None. **S. Naaman:** None. **A. Shmuel:** None.

Nanosymposium

481. Oscillations and Synchrony

Location: 152B

Time: Tuesday, November 18, 2014, 8:00 AM - 10:45 AM

Presentation Number: 481.03

Topic: B.09. Network Interactions

Support: NIH Grant 5R21MH093858-02

Title: Functional connectivity arises from a slow rhythmic mechanism

Authors: ***W. J. BENTLEY**[1], J. LI[1], A. SNYDER[2], M. RAICHLE[2], L. SNYDER[1];
[1]Anat. and Neurobio., [2]Radiology, Washington Univ., Saint Louis, MO

Abstract: Resting-state functional-connectivity MRI (rs-fcMRI) analyses have provided tremendous insight into the brain's functional architecture. These analyses evaluate the inter-regional correlation pattern of slow (0.01-0.1 Hz) task-independent fluctuations in the blood oxygen level dependent (BOLD) fMRI signal. Correlation structure has been used to describe the general topology of functional networks (functional connectivity), behaviorally relevant individual network differences, and network disruptions in disease. Despite widespread use and utility of these analyses, little is known about the origins of resting-state BOLD correlation. We used a high temporal-resolution measurement, oxygen polarography, to better characterize oxygen fluctuations and their correlation and gain insight into the driving mechanism. We simultaneously recorded oxygen fluctuations from two nodes in the default mode network (bilateral posterior cingulate) and two nodes in the visual/attention network (bilateral V3) in each of two resting macaques. Oxygen polarography provides a broader frequency range than possible with BOLD fMRI. Local oxygen fluctuation amplitude is well fit by a "$P = 1/f^{\alpha}$" spectrum model across a wide frequency range (0.001-20 Hz), where P is power, f is frequency and α is the exponent. BOLD fluctuations have been shown to have a $1/f^{\alpha}$ spectrum within the limited frequency range of BOLD fMRI. Moreover, we show that oxygen polarography captures inter-regional correlation, similar to BOLD correlation. Distinct from the broadband predominantly $1/f^{\alpha}$ spectrum of local fluctuations, correlation strength is only present in a restricted frequency window, with a clear peak at 0.06Hz. There is almost no correlation from 0.001 to 0.01 Hz or from 0.3 to 5 Hz. Synchrony and coherence are similarly band limited. Re-examing the power spectrum of local oxygen fluctuations, we find a deviation from a strict $1/f^{\alpha}$ fit. The profile of this deviation closely matches the frequency profile of the oxygen correlation. These two results suggest that there is a band-limited mechanism(s) driving interregional oxygen correlation that is distinct from the mechanism(s) driving the local $1/f^{\alpha}$ fluctuations. The fact that correlation is band limited is highly suggestive of a rhythmic or pseudo-oscillatory mechanism. Band-limited rhythms can reflect specific resonant mechanisms or circuits. Thus our results open up the possibility of a 'functional connectivity nucleus'.

Disclosures: W.J. Bentley: None. **J. Li:** None. **A. Snyder:** None. **M. Raichle:** None. **L. Snyder:** None.

Nanosymposium

481. Oscillations and Synchrony

Location: 152B

Time: Tuesday, November 18, 2014, 8:00 AM - 10:45 AM

Presentation Number: 481.04

Topic: B.09. Network Interactions

Support: Intramural Research Program of the National Institute of Neurological Disorders and Stroke, National Institutes of Health

Title: Electrophysiological and behavioral contributions to the resting-state fMRI signal

Authors: *C. CHANG[1], D. A. LEOPOLD[2], M. L. SCHÖLVINCK[3], X. LIU[1], H. MANDELKOW[1], J. H. DUYN[1];
[1]NIH (NINDS), Bethesda, MD; [2]NIH (NIMH), Bethesda, MD; [3]Ernst Strüngmann Inst. (ESI) for Neurosci. in Cooperation with Max Planck Society, Frankfurt am Main, Germany

Abstract: Spontaneous BOLD signal fluctuations and their spatial patterns of synchrony are widely used for investigating the functional organization of the brain. Yet, the neural basis of these fluctuations is not fully understood, fundamentally limiting the inferences one can draw from such data. Insight may be obtained by acquiring direct, invasive measurements of neural activity concurrently with fMRI. Here, using simultaneous electrophysiological and fMRI data recorded from awake macaques, we investigate frequency-specific contributions of local field potential (LFP) power fluctuations to the spontaneous fMRI signal. Furthermore, we examine the link between these signals and the behavioral state of the animal, as assessed by videos of eye opening/closure. LFP was measured from a single site in V1, and regressors for low (3-7.5Hz; "LF") and high (40-80Hz; "HF") frequency bands of the LFP were constructed by computing, within each fMRI TR, the mean power of the LFP signals in these bands. Using data from an infrared video camera, we constructed a binary “eye signal” indicating whether the eyes were open or closed, and both LFP and eye regressors were convolved with a standard hemodynamic response function prior to comparison with fMRI. The spatial dependence of low- and high-frequency LFP correlations with fMRI indicate distinct spatial signatures: HF power correlated most strongly within a network of visual cortex regions, while LF was correlated farther away from the recording site, consistent with findings that LF may reflect longer-range cortical processes. Results also support the notion that electrophysiological correlates of fMRI arise from processes involving multiple distinct frequency bands. Moreover, behavioral data suggest that the states of open and closed eyes are linked with both LFP and fMRI signal changes, contributing to their observed correlation.

Disclosures: C. Chang: None. **D.A. Leopold:** None. **M.L. Schölvinck:** None. **X. Liu:** None. **J.H. Duyn:** None. **H. Mandelkow:** None.

Nanosymposium

481. Oscillations and Synchrony

Location: 152B

Time: Tuesday, November 18, 2014, 8:00 AM - 10:45 AM

Presentation Number: 481.05

Topic: B.09. Network Interactions

Support: DHHS/NIH/NIMH, NINDS, NEI/IRP

Title: Transient inactivation of basal forebrain subregions shapes spontaneous fMRI correlations in the macaque

Authors: *J. N. TURCHI[1], C. CHANG[2], I. E. MONOSOV[3], K. SMITH[4], D. K. YU[4], F. Q. YE[4], C. ZHU[4], C. R. CORTES[5], M. MISHKIN[4], J. H. DUYN[2], D. A. LEOPOLD[4];
[1]LN, NIMH, NIH, Bethesda, MD; [2]NINDS, Bethesda, MD; [3]NEI, Bethesda, MD; [4]NIMH, Bethesda, MD; [5]NIAA, Bethesda, MD

Abstract: While the spatial pattern of correlations in spontaneous fMRI activity, sometimes called functional connectivity (FC), is widely used and interpreted in the study of the human brain, its physiological origins and the basis of its specific spatial correlations are largely unknown. One possibility, not previously investigated, is that the underlying slow hemodynamic fluctuations, along with their shared structure across different cortical areas, are shaped by long-range input from a common source. Here, we investigate the hypothesis that the basal forebrain (BF) provides such input, and thus plays a role in shaping FC. Groups of large cholinergic and GABAergic neurons in the BF are known to project widely throughout the cerebral cortical mantle. This input is thought to modulate cortical excitability locally, thus influencing brain functions as diverse as stimulus processing, motivation, and learning. We investigate the BF contribution to FC at rest by measuring changes to spontaneous fMRI fluctuations after reversibly inactivating components of the basal forebrain, including the nucleus basalis of Meynert. We infused muscimol (18 mM-44mM, 2.46 μl) into several portions of the BF, comparing patterns of FC following inactivation to those following vehicle infusions. The contrast agent gadolinium was included to confirm targeting. For functional scanning, intravenous MION was administered and two to three resting scans, each lasting thirty minutes, were collected (EPI, 1.5 mm isotropic volume, TR = 2.5 s). Non-neural components of the fMRI time series were minimized using CO_2 and video images collected during the scan. Unilateral inactivation of the basal forebrain had a strong impact on spontaneous activity, and hence FC, in the injected hemisphere. In control sessions for which saline was injected, spatial independent component analysis (ICA) revealed functional “networks”, which were in all cases bilateral and corresponded well to the known resting state networks in the macaque. However, following inactivation, many networks became independent in the left and right cerebral cortex, indicating a hemispheric uncoupling. Furthermore, a comparison of the fMRI time series correlations across all pairs of voxels within and between hemispheres revealed that the injected hemisphere was significantly weaker in its FC than the uninjected side. Our data demonstrate that resting state FC

is, at least in part, shaped by long-range, common input projections arising from the basal forebrain.

Disclosures: **J.N. Turchi:** None. **C. Chang:** None. **I.E. Monosov:** None. **K. Smith:** None. **D.K. Yu:** None. **F.Q. Ye:** None. **C. Zhu:** None. **C.R. Cortes:** None. **M. Mishkin:** None. **J.H. Duyn:** None. **D.A. Leopold:** None.

Nanosymposium

481. Oscillations and Synchrony

Location: 152B

Time: Tuesday, November 18, 2014, 8:00 AM - 10:45 AM

Presentation Number: 481.06

Topic: B.09. Network Interactions

Support: NSERC (RGPIN 375457-09)

Human Frontier Science Program (RGY0080/2008)

Title: Performances of functional network modeling methods in minimizing false positive and maximizing true positive connections

Authors: D. A. DAWSON[1], *J. D. MENDOLA[2], A. SHMUEL[1];
[1]MNI, McGill Univ., Montreal, QC, Canada; [2]Dept Ophthalmol, McGill Univ., Montreal, QC, Canada

Abstract: Various network models are being implemented in order to study functional brain networks in humans. These models often utilize Blood Oxygenated Level Dependent functional Magnetic Resonance Imaging (BOLD fMRI) data because of relatively high spatial resolution. One concern in such network analyses is eliminating the presence of false positive results. Among the most common approaches is thresholding the connectivity measures, however this can be arbitrary and requires a careful choice in the severity of the threshold; a very strict threshold can also eliminate true positive results. As of yet, there are no good standards for setting this threshold without prior knowledge of the system of study. Because of this, we aim to evaluate the ability of various promising models in terms of their ability to minimize false positives and maximize true positives, using common statistical significance thresholding. We evaluate the models using resting-state fMRI data from the human visual cortex. We compare functional connectivity from humans to the gold standard tracer-based structural connectivity studied in primates. Multivariate models were shown to be the only option in order to

differentiate between direct and indirect connectivity in this local network. Partial correlation and Bayesian network models PC and GES most reliably predicted expected connections while not predicting unexpected connections, with Partial correlation showing the most flexibility in terms of number of nodes in the network and whether a global signal is regressed. In a larger network, ICOV_5 (Regularized Inverse Covariance) performs better than the models which were more broadly successful. In general, network predictions were closer to expectations when the global signal was regressed out of the ROI timeseries. Bivariate models such as correlation were only able to produce useable network predictions when strict thresholding was done. We recommend the use of multivariate models for future study of unknown brain networks, as these will be most reliable in providing only direct connections between network nodes.

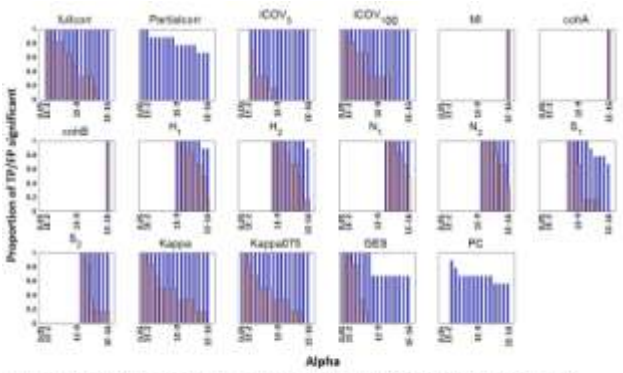

Fig. 1. Proportion of true and false positive connections found for each of the models tested at various significance thresholds (alpha). Blue bars are the true positives, and red bars are the false positives. In this 9 node network within a quadrant of the visual cortex (data with the global signal removed), there are 9 true positives tested for and 6 false positives. Starting significant p-values are with an alpha of 0.05 which is adjusted for multiple comparisons using the false discovery rate correction.

Disclosures: **D.A. Dawson:** None. **A. Shmuel:** None. **J.D. Mendola:** None.

Nanosymposium

481. Oscillations and Synchrony

Location: 152B

Time: Tuesday, November 18, 2014, 8:00 AM - 10:45 AM

Presentation Number: 481.07

Topic: B.09. Network Interactions

Support: R01NS078223

R01NS084028

P01NS080675

Title: Spontaneous fad dynamics reveal functional connectivity patterns in mice

Authors: P. W. WRIGHT[1], A. Q. BAUER[1], *J. P. CULVER[2];
[1]Washington Univ., St. Louis, MO; [2]Radiology, Washington Univ. in St Louis, Saint Louis, MO

Abstract: Functional connectivity (FC) describes the functional relationships within brain networks. The most well known method for measuring FC, fcMRI, analyzes spatio-temporal correlations in low frequency (0.009-0.08 Hz) spontaneous brain activity; this method has already been shown to be exquisitely sensitive to the progression of a wide array of neuropathologies. An analogous approach, optical intrinsic signal (fcOIS) imaging, has recently been extended to mice [1]. Here we extend FC imaging beyond hemodynamic contrasts to metabolite dynamics, in particular to that of flavin adenine dinucleotide (FAD). Whereas the hemodynamic response to neural activity is indirect via a multi-step neurovascular coupling process, optical signals via metabolites (e.g. FAD) allow for imaging of cellular mechanisms known to be part of individual synaptic potential events. FAD is excited in the blue range of the visible light portion of the electromagnetic spectrum ($\lambda \approx 460$nm) and subsequently fluoresces green ($\lambda \approx 520$nm). The increased availability of calcium following neuronal action potentials allows for aerobic energy metabolism to occur, leading to the oxidation of flavoproteins and consequently, the potential for autofluorescence. Though previous studies have used flavoprotein autofluorescence imaging to characterize metabolic activity in studies of cortical plasticity and somatosensory evoked responses, no studies have assessed the utility of FAD autofluorescence as an index for functional connectivity analysis. Wild-type Swiss Webster mice were imaged transcranially with sequential illumination provided by three collimated, collinear LEDs. A blue LED ($\lambda \approx 470$nm) was used for FAD excitation, while red ($\lambda \approx 625$nm) and green ($\lambda \approx 530$nm) LEDs were used in combination to construct the OIS signal and derive relative changes in oxyhemoglobin concentration. Mechanical stimulation of the right hindpaw was used to quantitatively evaluate the spatial and temporal resolution of the FAD signal as compared to OIS. Evaluation of spontaneous FAD signals demonstrated their ability in mapping FC patterns directly from metabolite dynamics. Mouse-specific FC mapping could provide a link between molecular level mouse models of disease and their corresponding human analog. Moreover, simultaneous FAD and OIS imaging can provide alternative, complimentary views of neuronal mechanisms underlying diseases impacting neurovascular coupling.

Disclosures: P.W. Wright: None. **J.P. Culver:** None. **A.Q. Bauer:** None.

Nanosymposium

481. Oscillations and Synchrony

Location: 152B

Time: Tuesday, November 18, 2014, 8:00 AM - 10:45 AM

Presentation Number: 481.08

Topic: B.09. Network Interactions

Support: CIHR Doctoral Research Award

NIH TR01 GM104948

NIH DP1 OD003646

NIH DP2OD006454

Title: Predicting behavioral state from neural dynamics during light sedation and general anesthesia

Authors: *L. D. LEWIS[1], R. A. PETERFREUND[2], L. S. AGLIO[3], P. G. HARRELL[2], E. N. ESKANDAR[2], L. F. BARRETT[4], S. S. CASH[5], E. N. BROWN[6], P. L. PURDON[5];
[1]Brain and Cognitive Sci., MIT, Cambridge, MA; [2]Massachusetts Gen. Hosp., Boston, MA; [3]Brigham and Women's Hosp., Boston, MA; [4]Northeastern Univ., Boston, MA; [5]Harvard Med. Sch., Boston, MA; [6]Harvard-MIT, Boston, MA

Abstract: Many anesthetic drugs produce mild sedation at low doses, and cause profound unconsciousness when administered at higher doses. Wake, sedation, and general anesthesia are dramatically different behavioral states, but the neural dynamics that cause this progressive disruption of cognitive function are not well understood. We studied intracranial electrocorticography (ECoG) recordings from patients undergoing an anesthetic induction prior to clinically indicated surgery. Patients performed a simple auditory task while receiving a gradual infusion of propofol, a widely used anesthetic, enabling us to study the relationship between propofol-induced ECoG dynamics and task performance. We found that patients' arousal state decreased gradually during the induction: they initially became sedated, reflected by an increase in reaction time, and later became fully unresponsive to auditory stimuli. We characterized how evoked auditory responses were altered during sedation, and found that while event-related potentials (ERPs) still occurred during unconsciousness, ERP amplitudes were reduced on trials where patients failed to respond. We then developed a machine learning-based classification method that used spectral features of the ECoG recordings to predict patients' behavioral state, defined as the ability to respond to the auditory stimulus. We found that activity in a subset of cortical regions was sufficient to predict behavioral state at significantly above-chance levels, and that slow wave (0.1-4 Hz), alpha (10-15 Hz) and gamma (25-50 Hz) power dynamics in these regions were particularly relevant for inferring behavioral state. However, the effects on both the ERPs and the ongoing ECoG oscillations were subtle, in contrast to striking changes that occurred at deeper levels of anesthesia. This approach may be useful clinically to infer a patient's state of consciousness and anesthetic needs during surgery. In addition, these

results provide insight into the set of neural dynamics that are required for normal waking function, and the circuits that are disrupted by general anesthesia.

Disclosures: **L.D. Lewis:** None. **R.A. Peterfreund:** None. **L.S. Aglio:** None. **P.G. Harrell:** None. **E.N. Eskandar:** None. **L.F. Barrett:** None. **S.S. Cash:** None. **E.N. Brown:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Technology licensing agreement with Masimo. **P.L. Purdon:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Technology licensing agreement with Masimo.

Nanosymposium

481. Oscillations and Synchrony

Location: 152B

Time: Tuesday, November 18, 2014, 8:00 AM - 10:45 AM

Presentation Number: 481.09

Topic: B.09. Network Interactions

Support: CIHR

JS McDonnell Foundation

Title: Network structure supports stable functional connectivity of homotopic regions across time and conditions

Authors: ***K. SHEN**[1], B. MISIC[1,2], G. BEZGIN[1], M. BUSCHKUEHL[3], P. J. DELDIN[4], R. HUTCHISON[5], S. M. JAEGGI[3], E. KROSS[4], S. PELTIER[4], S. EVERLING[6,7], J. JONIDES[4], M. G. BERMAN[8], A. R. MCINTOSH[1,2];
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Abstract: Functional connectivity (FC) between homotopic regions is known to be stronger than that between other interhemispheric (i.e., heterotopic) regions. The strength of homotopic FC is thought to be due to their shared functional role as well as to their structural connectivity: homotopic anatomical projections represent the majority of callosal fibers. Given recent findings that FC is highly variable over time and across conditions, it remains to be determined whether

strong homotopic FC is consistently present under such circumstances. In this study, we tested the hypotheses that 1) homotopic FC is more stable across time and conditions than heterotopic FC and, 2) stability in homotopic FC is conferred by the underlying anatomical connectivity. Functional magnetic resonance imaging (fMRI) data were obtained from both humans (n=17) and monkeys (Macaca fascicularis; n=6). Both species underwent resting-state fMRI scans (humans: 3 8-min scans each; macaques: 2 10-min scans each, obtained under light anaesthesia). Human participants additionally underwent an 8-min induced rumination scan, where participants were asked to ruminate/think about a negative autobiographical memory. In addition, structural connectivity of the macaque brain was determined by querying the online CoCoMac database. Consistent with previous findings, inter-hemispheric FC between homotopic regions was significantly stronger in both humans and macaques. To investigate condition stability, a multivariate Partial Least Squares (PLS) analysis was performed to determine the extent to which each functional connection changed between conditions/scans. In both species, homotopic functional connections were significantly more stable across conditions as compared to heterotopic and intrahemispheric connections. To investigate temporal stability, FC was calculated in sliding windows. Temporal stability of each connection was defined as the average autocorrelation coefficient across time lags. In both species, homotopic FC had significantly greater stability than other types of connections. In the macaque, homotopic pairs with direct anatomical projections exhibited significantly greater stability than homotopic pairs without direct anatomical projections, suggesting that the temporal stability of homotopic FC is at least partly mediated by direct callosal projections. Our data indicate that homotopic FC is not only stronger, but more stable across time and conditions. Importantly, we show how direct structural connectivity is a crucial determinant of the dynamics of interhemispheric functional interactions.

Disclosures: **K. Shen:** None. **B. Misic:** None. **G. Bezgin:** None. **M. Buschkuehl:** None. **P.J. Deldin:** None. **R. Hutchison:** None. **S.M. Jaeggi:** None. **E. Kross:** None. **S. Peltier:** None. **S. Everling:** None. **J. Jonides:** None. **M.G. Berman:** None. **A.R. McIntosh:** None.

Nanosymposium

481. Oscillations and Synchrony

Location: 152B

Time: Tuesday, November 18, 2014, 8:00 AM - 10:45 AM

Presentation Number: 481.10

Topic: B.09. Network Interactions

Title: Cortex-specific correlation between locally recorded slow oscillations and BOLD fMRI

Authors: *M. SCHWALM[1], F. SCHMID[2], L. WACHSMUTH[2], C. FABER[2], A. STROH[1]; [1]Focus Program Transl. Neurosci. (ftn) & Inst. for Microsc. Anat. and Neurobiol., Johannes Gutenberg Univ., Mainz, Germany; [2]Dept. of Clin. Radiology, Univ. of Münster, Münster, Germany

Abstract: Slow oscillations reflect brain state transitions, occurring spontaneously in slow wave sleep and under light anesthesia. Here, we combined spatio-temporally precise optical Ca2+ recordings in rat somatosensory cortex with blood oxygenation level-dependent (BOLD) fMRI of the whole brain. Upon injection of the Ca2+ indicator Oregon Green BAPTA-1, we achieved a defined area of staining, typically covering a sphere with a diameter of 600 µm. Unlike electric population recordings, optical recordings are confined to monitoring activity in the stained region, thereby excluding e.g. sub-cortical activity in cortical recordings. Using this setup, we monitor suprathreshold neuronal spiking activity alongside 9.4 T fMRI. Under light isoflurane anesthesia, the optic-fiber-based Ca2+ recordings allowed us to monitor spontaneous slow oscillations on population level in real time, which are associated with Up/Down state transitions in cortical and thalamic neurons. Optic-fiber-based Ca2+ recordings revealed typical slow Ca2+ waves with a frequency ranging at 16 - 20 waves/min. Next, we asked whether these large-amplitude oscillations in mean neuronal firing activity are reflected in the BOLD signal at rest. We developed an amplitude- and duration-based algorithm capturing Up/Down state transitions in the Ca2+ traces resulting in a binary vector defining either Up or Down state for each time point of the recording period. Using this vector as a regressor for an event-related analysis of the simultaneously acquired fMRI data, with onset times and durations of the Up states as events, we discovered a spatially confined correlation between spontaneous slow oscillatory activity and the BOLD signal: the entire cortex oscillates in synchrony with the slow oscillations that were recorded locally in S1. These results of a region-specific recruitment in slow wave sleep suggest a prominent role of slow oscillations in cortical signal processing.

Disclosures: **M. Schwalm:** None. **F. Schmid:** None. **L. Wachsmuth:** None. **C. Faber:** None. **A. Stroh:** None.

Nanosymposium

481. Oscillations and Synchrony

Location: 152B

Time: Tuesday, November 18, 2014, 8:00 AM - 10:45 AM

Presentation Number: 481.11

Topic: B.09. Network Interactions

Support: NSF CRCNS 1131850

Brown Institute for Brain Science/Norman Prince Neuroscience Institute New Frontiers Fund

Title: Neocortical beta (15-20hz) events emerge from dendritic integration of synaptic bursts to distinct cortical layers: Converging evidence from humans, modeling, monkey and mouse

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Abstract: Beta frequency rhythms (15-29 Hz) are one of the most dominant activities measured non-invasively in humans with technologies such as MEG and EEG. Beta rhythms are implicated to be important in many cognitive and motor tasks, including selective attention, perception, and motor planning and initiation. They are also disrupted in many neuropathologies, most notably Parkinson's Disease, in which treatments that alleviate symptoms also reduce neocortical beta. Despite their prevalence, an understanding of their precise role in information processing is unknown. Key to understanding the computational importance of beta is to understand the underlying cellular and network level generators of this rhythm. Many theories have been proposed for the origin of neocortical beta, including direct beta frequency synaptic drive from subcortical thalamic and basal ganglia structures. Here we propose an alternative theory for beta measured with MEG/EEG. Our theory suggests that beta oscillations in these signals, which typical manifest as one to two cycle beta "events" and not continuous oscillations, emerge from the integration of nearly-synchronous bursts of excitatory synaptic drive to granular and supragranular cortical layers that create alternating current flow of opposing polarity within the apical dendrites of long and spatially aligned cortical pyramidal neurons. This produces an oscillatory response with a period in the beta band. Importantly, the supragranular burst of excitatory drive is stronger than the granular burst and lasts ~40ms. This theory was derived from a computational neural model designed to accurately reproduce the electromagnetic physics of the MEG/EEG current source signal. Our model simulations reproduce many quantifiable features in beta rhythms from primary somatosensory cortex (SI) measured with MEG, and these results are supported by initial data from laminar recordings in SI of awake monkeys and anesthetized mice. Specifically, in the animal data, we observed beta events in the local field potential (LFP) signal from granular layers that have consistent polarity and temporal features to beta events in the human and model data. Moreover, current source density analysis overlaid on the LFP beta signal shows spatially aligned sinks, consistent with excitatory synaptic drive to granular and supragranular layers, as directly predicted by the model. We are currently exploring the presynaptic sources of these excitatory burst events. Our results provide a novel interpretation of the mechanistic origin of beta that can help guide future studies on beta's role in information processing and motor control.

Disclosures: **M.A. Sherman:** None. **S. Haegens:** None. **S. Lee:** None. **C. Thorn:** None. **C.E. Schroeder:** None. **C.I. Moore:** None. **S.R. Jones:** None.

Nanosymposium

482. Aggregation of Amyloid, Tau, and Other Proteins

Location: 152A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 482.01

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

Title: The response of Aβ oligomers found in non-human primate cerebrospinal fluid following treatment with a gamma secretase inhibitor, MK-0752

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Abstract: Alzheimer's disease (AD) is the most common neurodegenerative dementia in the elderly. The pathognomonic senile plaques found in the brains of AD patients are comprised largely of aggregated amyloid-β (Aβ) peptide. Several forms of soluble oligomeric Aβ have also been identified and increasing evidence suggests that oligomers are toxic to neurons. Therapeutic approaches to reduce Aβ have been advanced, including secretase inhibitors that target Aβ formation from the amyloid precursor protein and immunotherapy approaches to facilitate amyloid clearance/neutralization from the brain. Here, we examined the response of Aβ oligomers in rhesus CSF following treatment with the gamma secretase inhibitor (GSI) MK-0752. An Aβ oligomer-specific assay was developed (J. Neuroscience 34:2884), detecting Aβ oligomers comprised of either synthetic standards, or endogenous oligomers from cerebrospinal fluid (CSF) or from histologically confirmed human AD and healthy control subjects. Using this assay, a robust 3-5 fold increase in Aβ oligomers was observed in the CSF of AD patients (N=63) vs. age-matched healthy controls (N=54), as well as from SEC-fractionated aqueous supernatants of AD compared to non-AD cortex. In addition to detecting Aβ oligomers in human CSF, these species are also detected in non-human primate (NHP) rhesus CSF at concentrations similar to normal human. We studied the response of NHP CSF oligomers to acute, single dose treatment with MK-0752 and the relationship of oligomer changes to changes in Aβ monomers. Aβ oligomers are reduced in a time-dependent manner following secretase inhibitor treatment however the overall response kinetics and magnitude of response differs compared with either Aβ40 or Aβ42 monomers. In addition to the potential utility of this Aβ oligomer assay as a

diagnostic tool to detect AD, our data suggest it can be applied as a pharmacodynamic readout of secretase inhibition in NHP CSF.

Disclosures: **M.J. Savage:** A. Employment/Salary (full or part-time):; Merck and Company. **J. Kalinina:** A. Employment/Salary (full or part-time):; Merck and Company. **M.S. Michener:** A. Employment/Salary (full or part-time):; Merck and Company. **B.E. Smith:** A. Employment/Salary (full or part-time):; Merck and Company.

Nanosymposium

482. Aggregation of Amyloid, Tau, and Other Proteins

Location: 152A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 482.02

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

Support: Georgetown University

Merck and Co

Title: Autophagic intraneuronal Aβ1-42 clearance requires normal Tau function and modulates extracellular plaque deposition

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Abstract: Tau is an axonal protein that binds to and regulates microtubule function. Hyper-phosphorylation of Tau, as occurs in Alzheimer's disease, leads to reduced Tau binding to microtubules and it is associated with β-amyloid deposition and neurodegeneration. Paradoxically, Tau reduction may prevent β-amyloid pathology, raising the possibility that Tau may be involved in Aβ clearance. The current studies investigated the role of Tau in intraneuronal Aβ1-42 clearance and the subsequent effect on plaque deposition. Lentiviral expression of Aβ1-42 led to time-dependent increase in Tau hyper-phosphorylation (p-Tau) in wild type mouse primary neurons. Inhibition of either the proteasome or autophagy led to partial reductions of Aβ1-42 degradation and increased p-Tau generation, suggesting involvement of both degradation systems in the intracellular clearance of Aβ1-42. Interestingly, Tau deletion impaired Aβ clearance via autophagy, but not by the proteasome, while introduction of wild type human Tau into Tau-/- mice restored autophagic clearance of Aβ1-42, suggesting that exogenous

Tau expression can support autophagic Aβ1-42 clearance. Tau deletion impaired autophagic flux and resulted in Aβ1-42 accumulation in pre-lysosomal autophagic vacuoles, affecting Aβ1-42 deposition into the lysosome. This autophagic defect was associated with decreased intraneuronal Aβ1-42 and increased plaque load in Tau-/- mice. Nilotinib, an Abl tyrosine kinase inhibitor that promotes autophagic clearance mechanisms, reduced Aβ1-42 only when exogenous human Tau was expressed in Tau-/- mice. These studies demonstrate that autophagic intraneuronal Aβ1-42 clearance requires normal Tau function to modulate extracellular plaque.

Disclosures: C.E. Moussa: None. **I. Lonskaya:** None. **M. Hebron:** None.

Nanosymposium

482. Aggregation of Amyloid, Tau, and Other Proteins

Location: 152A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 482.03

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

Support: CNPQ

FAPERJ

CAPES

Canadian Institutes for Health Research

Canada Research Chair Program

Title: Alzheimer's disease-like pathology induced by Abeta oligomers in non- human primates

Authors: *L. FORNY GERMANO[1], N. M. LYRA E SILVA[2], A. F. BATISTA[2], J. BRITO-MOREIRA[2], M. GRALLE[2], S. BOEHNKE[4], B. C. COE[4], A. LABLANS[4], S. A. MARQUES[5], A. B. MARTINEZ[3], W. L. KLEIN[6], J.-C. HOUZEL[3], S. T. FERREIRA[2], D. P. MUNOZ[4], F. G. DE FELICE[1];
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Abstract: Alzheimer's disease (AD) is a devastating neurodegenerative disorder and a major medical problem. Here, we have investigated the impact of amyloid-beta (Abeta) oligomers, AD-related neurotoxins, in the brains of rats and adult non-human primates (cynomolgus macaques). Soluble Abeta oligomer are known to accumulate in the brains of AD patients and correlate with disease-associated cognitive dysfunction. When injected into the lateral ventricle of rats and macaques, Aβ oligomers diffused in the brain and accumulated in several regions associated with memory and cognitive functions. Cardinal features of AD pathology, including synapse loss, tau hyperphosphorylation and astrocyte activation, were observed in regions of the macaque brain where Abeta oligomers were abundantly detected. Most importantly, oligomer injections induced AD-type neurofibrillary tangle formation in the macaque brain. These outcomes were specifically associated with Abeta oligomers, as fibrillar amyloid deposits were not detected in oligomer-injected brains. Human and macaque brains share significant similarities in terms of overall architecture and functional networks. Thus, generation of a macaque model of AD that links Aβ oligomers to tau and synaptic pathology has the potential to greatly advance our understanding of mechanisms centrally implicated in AD pathogenesis. Furthermore, development of disease-modifying therapeutics for AD has been hampered by the difficulty in translating therapies that work in rodents to humans. Therefore, generating a reliable macaque model is likely to enable development of new AD therapies.

Disclosures: L. Forny Germano: None. **N.M. Lyra e Silva:** None. **A.F. Batista:** None. **J. Brito-Moreira:** None. **M. Gralle:** None. **S. Boehnke:** None. **B.C. Coe:** None. **A. Lablans:** None. **S.A. Marques:** None. **A.B. Martinez:** None. **W.L. Klein:** None. **J. Houzel:** None. **S.T. Ferreira:** None. **D.P. Munoz:** None. **F.G. De Felice:** None.

Nanosymposium

482. Aggregation of Amyloid, Tau, and Other Proteins

Location: 152A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 482.04

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

Title: Intracerebral injection of preformed synthetic tau fibrils initiates widespread tauopathy as well as neuronal loss in the brains of tau transgenic mice

Authors: *D. W. MOECHARS[1], E. PEERAER[1], A. BOTTELBERGS[1], K. BRUNDEN[2], V. LEE[2], J. TROJANOWSKI[2], J. KEMP[1];
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Pathology and Laboratory,, Ctr. for Neurodegenerative Dis. Research, Inst. on Aging, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA

Abstract: Background: Neurofibrillary tangles (NFTs) composed of hyperphosphorylated fibrillized tau are found in numerous tauopathies including Alzheimer's Disease (AD). Increasing evidence suggests that tau pathology can be transmitted from cell-to-cell; however, the mechanisms involved in the initiation of tau fibrillization and spreading of disease linked to progression of tau pathology are poorly understood. Methods: Intracerebral injections of preformed synthetic tau fibrils (PFFs) into the hippocampus or frontal cortex of 3 months old tau transgenic (Tg) mice expressing mutant human P301L tau. Results: We show here that intracerebral injections of preformed synthetic tau fibrils (PFFs) into the hippocampus or frontal cortex of young tau transgenic (Tg) mice expressing mutant human P301L tau induces tau hyperphosphorylation and aggregation around the site of injection, as well as a time-dependent propagation of tau pathology to interconnected brain areas distant from the injection site. Both the injection site and the nature of the seed affect the distribution and characteristics of the resulting tau pathology. Furthermore, we show that injection of tau PFFs into the hippocampus induces selective loss of CA1 neurons. Conclusions: Together, our data confirm previous studies on the seeded induction and the spreading of tau pathology in a different tau Tg mouse model and reveals for the first time neuronal loss after intracerebral injection of tau PFFs in tau Tg mouse brain. These results further validate the utility of the tau seeding model in studying disease transmission, and provide a more complete in vivo tauopathy model with associated neurodegeneration which can be used to investigate the mechanisms involved in tau aggregation and spreading, as well as aid in the search for disease modifying treatments for AD and related tauopathies.

Disclosures: D.W. Moechars: A. Employment/Salary (full or part-time):; Janssen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Janssen. **E. Peeraer:** A. Employment/Salary (full or part-time):; Janssen. **A. Bottelbergs:** A. Employment/Salary (full or part-time):; Janssen. **K. Brunden:** None. **V. Lee:** None. **J. Trojanowski:** None. **J. Kemp:** A. Employment/Salary (full or part-time):; Janssen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Janssen.

Nanosymposium

482. Aggregation of Amyloid, Tau, and Other Proteins

Location: 152A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 482.05

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

Support: Zenith Fellows Award (C.G.) from the Alzheimer's Association

NIA Grant AG014706

NIA Grant AG027141

Title: Presence of calbindin-d_{28k} retards the process of tangle formation within basal forebrain cholinergic neurons in alzheimer's disease

Authors: S. S. AHMADIAN, M. PETERSON, S. WEINTRAUB, E. BIGIO, M. MESULAM, *C. GEULA;
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Abstract: The reasons for selective vulnerability of distinct neuronal populations in neurodegenerative disorders are poorly understood. We have used the basal forebrain cholinergic neurons (BFCN), which are selectively vulnerable in a number of neurodegenerative disorders associated with aging, including Alzheimer's disease (AD), to investigate the basis of the early and selective vulnerability to these neurons to tangle formation and degeneration in AD. Previously we showed that the majority of BFCN in the human brain contain the calcium binding protein calbinding-D_{28K} (CB) and that a large proportion of BFCN lose their CB in the course of normal aging. Importantly, the BFCN which degenerate in AD are those that lack CB. In this study, we investigated the relationship between the presence of CB in the BFCN and the process of tangle formation. We used AD brains obtained from the Brain Bank of the Northwestern University Alzheimer's Disease Center. Sections spanning the entire nucleus basalis (Ch4) component of BFCN were used to investigate the presence of neurofibrillary tangles in the CB-positive compared with CB-negative BFCN employing double immunohistochemistry with antibodies to CB and tau epitopes that appear early (tau oligomer complex-1 [TOC-1]), intermediate (AT8 and PHF1) or late (truncated tau MN423) in the process of tangle formation. Unbiased stereological methods were used for quantitation. A very small percentage (0 - 3.7%) of CB immunoreactive BFCN contained pre-tangles / tangles. Similarly, very small percentages (0 - 5%) of the total BFCN pre-tangles / tangles were in CB immunoreactive neurons. The number of CB-positive BFCN which contained tau immunoreactivity was highest for the early epitope (TOC1), decreased with intermediate epitopes, and was lowest for the latest appearing epitope (MN423, $p<0.05$). The percentage of CB immunoreactive BFCN containing the TOC1 epitope was also significantly higher than for all other epitopes ($p<0.01$-0.001), and the percentage containing the late appearing epitope was significantly lower than the intermediate epitopes ($p<0.01$-0.001). Similar results were obtained when comparing the percentage of total tau immunoreactive BFCN which were CB-positive. In conclusion, age-related loss of CB appears to render the BFCN vulnerable to tau accumulation and tangle formation. Conversely,

the presence of CB in BFCN retards the process of tangle formation. Therefore, at least one mechanism through which CB protects the BFCN from neurodegenerative processes is through significant retardation of tangle formation, most likely via its primary function in regulating intracellular calcium concentrations.

Disclosures: **S.S. Ahmadian:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Lster Binder PhD. **C. Geula:** None. **M. Peterson:** None. **S. Weintraub:** None. **M. Mesulam:** None. **E. Bigio:** None.

Nanosymposium

482. Aggregation of Amyloid, Tau, and Other Proteins

Location: 152A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 482.06

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

Support: The Alzheimer's Drug Discovery Foundation

The Cullen Trust

The Mitchell Center for Neurodegenerative Diseases

Title: Tau oligomers from Alzheimer's disease and Traumatic Brain Injury induce toxicity in Htau mice

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Abstract: Tau aggregation is a pathological feature of numerous neurodegenerative disorders, including Alzheimer's disease (AD), as well as Traumatic Brain Injury (TBI). There is no effective treatment for AD and disease burden continues to increase. TBI not only induces cognitive changes affecting millions of people, but also leads to increased incidence of neurodegeneration later in life. Growing evidence from our lab and others suggests that tau neurofibrillary tangles are not responsible for toxicity, but rather the oligomeric forms of tau initiate the onset and spread of disease. We have shown increased levels of tau oligomers in both AD brains and TBI rodent model brains. Using immunoprecipitation, we isolated tau oligomers from AD, fluid percussion injured rat and blast injured mouse brains. Oligomers were characterized biochemically and morphologically by atomic fluorescence microscopy and were

injected bilaterally in the hippocampi of mice overexpressing human tau (Htau mice). Mice were cognitively evaluated using novel object recognition and Y-maze tasks and brains were collected following testing for analysis. We found that tau oligomers, similar to those found in Alzheimer's disease, form as a result of brain injury in two different rodent models of TBI and brain-derived tau oligomers injected in Htau mice accelerated the onset of cognitive deficits. Biochemical and immunohistochemical analysis of mice injected with oligomers is ongoing. Tau oligomers are likely the most toxic species of tau in neurodegenerative disease and these results suggest that they play an important role in the toxicity underlying TBI as well, suggesting that they may be a viable therapeutic target in TBI and in preventing the increased acquisition of neurodegenerative disease.

Disclosures: J. Gerson: None. **D. Castillo-Carranza:** None. **U. Sengupta:** None. **C. Lasagna-Reeves:** None. **R. Kayed:** None.

Nanosymposium

482. Aggregation of Amyloid, Tau, and Other Proteins

Location: 152A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 482.07

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

Title: Inhibitors of glutaminyl cyclase (QC) which is up-regulated early in Alzheimer's Disease (AD) block toxic pGlu-Abeta formation and are save in early clinics

Authors: *H. U. DEMUTH[1], S. F. SCHILLING[1], I. LUES[2], K. GLUND[2];
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Abstract: AD is characterized by neuron loss and neuroinflammation. Although N-truncated and in particular N-pyroglutamated Aβ-peptides (pEAβ) are known as prominent constituents of plaques in AD brain, their importance was overseen for some time and pathways leading to their formation not understood. Because of their abundance, resistance to proteolysis, such N-terminally truncated and modified peptides are likely to be important for initiation of pathological cascades leading to AD. Our recent work uncovers, that the N-terminal pE-formation is catalyzed by glutaminyl cyclase (QC) in vivo. Target validation - QC expression was found upregulated in the cortex of individuals with AD and correlated with the appearance of pE-modified Aβ. Oral applications of QC inhibitors resulted in reduced pE3Aβ42 burden, but also in the attenuation of total Aβ in transgenic models of AD. Besides showing a diminished

plaque formation and gliosis, we found improved performance in context memory and spatial learning tests. These observations led to the hypothesis that pEAβ can seed Aβ oligomerization by self- and co-aggregation with other monomeric Aβ species. In recent in vitro, in situ and in vivo studies amounts of less than 100nM pE3Aβ42 generated cytotoxic oligomers which are over 20 fold more stable than oligomers of the classical full-length Aβ-peptides. Such mixed pE3Aβ42-oligomers propagate their toxic structure in a prion-like manner. Moreover, the neurotoxicity unfolds to be strictly tau-dependent in cell culture as well as in animal models. Specific neuronal expression of pEAβ provides in vivo evidence for profound pEAβ neurotoxicity and gliosis. Also, such toxic oligomers impair LTP, synaptic plasticity and behavior1,2,3. Drug identification - hence, a QC-inhibitor discovery program was launched, which has entered the regulatory phase 2010. PQ912 is a QC inhibitor for the treatment of AD and it is the first QC inhibitor in clinical development. Phase 1 single (SAD) and multiple ascending dose (MAD) trials of PQ912 in healthy volunteers were conducted in about 200 volunteers including elderly. The studies demonstrated that PQ912 is safe and well tolerated. Dose-proportional pharmacokinetics and a strong pharmacokinetic and pharmacodynamic relationship based on QC inhibition were observed in cerebrospinal fluid, showing an EC50 of 30nM. References 1. S. Schilling et al., Nature Medicine, 2008, 14, 1106-1111 2. J. Nussbaum et al. Nature, 2012, 485, 651-655 3. M. Morawski et al. J Alz. Dis. (2014) 39, 385-400 4. F. Weber et al. Neurodeg. Dis. (2013), 11, Suppl. 1 5. R. Black et al. Alzheimer’s Association International Conference (2013) Boston

Disclosures: **H.U. Demuth:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); stock, Probiodrug AG. **S.F. Schilling:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); stock, Probiodrug AG. **I. Lues:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Probiodrug AG. **K. Glund:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Probiodrug AG.

Nanosymposium

482. Aggregation of Amyloid, Tau, and Other Proteins

Location: 152A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 482.08

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

Support: BX001637

NS073899

Title: Chaperones control microtubule dynamics through tau

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Abstract: The interaction between the microtubule associated protein tau and chaperones is unusual simply because tau is a disease-associated intrinsically disordered protein (IDP) that lacks a defined structure. Here we sought to identify an evolutionary advantage for this specialized interaction. The constitutively expressed Hsp70 (Hsc70) is not only an initiator of the protein folding cycle, but can preserve tau levels in Alzheimer's disease and mouse models of tauopathy. Thus we generated a functionally inactive variant of Hsc70 to better understand the biological significance of the Hsc70/tau interface and examine why Hsc70 is so tightly coupled to tau proteostasis. NMR spectroscopy and biochemical characterization revealed that this Hsc70 variant resembled the ADP-bound conformer regardless of nucleotide binding, acting as a dominant negative form of Hsc70. This variant potently reduced tau levels via the proteasome in brain, which was in contrast to wildtype Hsc70 that preserved tau. Based on NMR spectroscopy showing similar binding domain specificity within tau for both wildtype and inactive Hsc70, we sought to determine how the interactome of functionally impaired Hsc70 differed from normal active Hsc70. Mass spectrometry and subsequent characterization revealed that DnaJs and Hsp90 were recruited to Hsc70 when its nucleotide exchange failed. Moreover, we found that this dominant negative form of Hsc70 was more associated with tubulin proteins. Based on these findings, we used live cell imaging to find that Hsc70 facilitates microtubule assembly but only when tau is present. But when Hsc70 is unable to exchange nucleotide, tau is degraded by via Hsp90, facilitating microtubule disassembly. This provides the first mechanism describing how chaperones use nucleotide exchange to regulate microtubule dynamics through tau, suggesting that tau is a functional bridge used by Hsc70 to control cytoskeletal changes. These data may provide insight into why rising levels of Hsc70 are associated with diseases involving microtubule instability and abnormal tau accumulation, such as Alzheimer's disease.

Disclosures: S.N. Fontaine: None. **S.M. Stevens:** None. **M. Zweckstetter:** None. **E.R.P. Zuiderweg:** None. **J.E. Gestwicki:** None. **C.A. Dickey:** None.

Nanosymposium

482. Aggregation of Amyloid, Tau, and Other Proteins

Location: 152A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 482.09

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

Support: The Alzheimer’s Drug Discovery Foundation

The Cullen Trust

The Mitchell Center for Neurodegenerative Diseases

Title: Tau oligomeric conformers: Implications for cell specificity and disease phenotypes

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Abstract: Background Tau pathology is implicated in a number of neurodegenerative diseases including Alzheimer’s disease. Studies repeatedly suggest that oligomeric forms of tau are the most toxic in disease. Moreover, the prion-like seeding and spreading of tau may depend upon oligomeric species. However, the mechanism by which native, unfolded tau monomer misfolds and aggregates to form oligomeric seeds is not entirely understood and evidence suggests that in other amyloid proteins, certain conformations of oligomers may be better able to seed further toxic aggregates. Therefore, it is of great importance to understand the ability of tau and its isoforms to form different oligomeric conformers. Methods We isolated and characterized tau oligomers from different neurodegenerative tauopathies. Moreover, we generated and characterized multiple anti-tau oligomer-specific mouse monoclonal antibodies (TOMAs), and used them to characterize different conformers of tau oligomers. Results Tau forms multiple oligomeric strains which can be distinguished biochemically with different TOMA clones. Individual TOMA clones recognize sequence-independent epitopes that are displayed by the different tau oligomer strains. Preliminary results from immunohistochemical analysis demonstrate the presence of at least two distinguishable subsets of tau oligomers. Different tau strains are capable of seeding in vitro. Strain-specific properties were also analyzed by Proteinase K digestion. Conclusion The ability of tau to form different oligomeric conformers may play a critical role in disease phenotype and progression. As individual TOMA clones display distinct preferences for different subsets of tau oligomers, these antibodies have great potential for use in personalized medicine for early diagnostics, the design of strain-specific

therapeutic approaches and for distinguishing polymorphisms and complexity of tau aggregation in different neurodegenerative tauopathies.

Disclosures: R. Kayed: None. **J. Gerson:** None. **U. Sengupta:** None. **M. Guerrero-Munoz:** None. **D. Castillo-Carranza:** None.

Nanosymposium

482. Aggregation of Amyloid, Tau, and Other Proteins

Location: 152A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 482.10

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

Support: NIH Grant R44AG032784 (GH)

NIH Grant R01ES016774 (MK)

VA Merit Review Grant 1I01RX000331 (MK)

NIH Grant R21AG042828 (VH)

Alzheimer's Association Grant (VH)

Title: Cathepsin B regulation of pGlu-Abeta and full-length Abeta related to memory deficits in an Alzheimer's disease mouse model

Authors: *V. Y. HOOK[1], J. YU[2], T. TONEFF[3], S. JACOBSEN[4,5], M. KINDY[2], G. HOOK[5]; [1]Univ. Calif, San Diego, LA JOLLA, CA; [2]Dept. Neurosciences, Med. Univ. of South Carolina, Ralph Johnson VA Med. Ctr., Charleston, SC; [3]Univ. of Calif., San Diego, La Jolla, CA; [4]AstraZeneca, Cambridge, MA; [5]American Life Sci. Pharmaceuticals, La Jolla, CA

Abstract: N-terminally modified pyroglutamate amyloid-beta peptides (pGlu-Abeta) and full-length (fl) Abeta are hypothesized to participate together in the development of memory deficits of Alzheimer’s disease (AD). pGlu-Abeta is abundant in AD brains and has been shown to initiate seeding of Abeta and pGlu-Abeta oligomers that are neurotoxic, leading to memory deficits of AD. Beta-secretase cleavage of amyloid-beta precursor protein (APP) produces flAbeta(1-40/42) which may then be converted to pGlu-Abeta, but demonstration of a requirement for beta-secretase in the production of pGlu-Abeta has not yet been shown. Therefore, this study examined effects of gene knockout (KO) of the BACE1 beta-secretase

compared to KO of the alternative beta-secretase cathepsin B (CatB) in the production of brain pGlu-Abeta in APPLon AD mice expressing APP-695 with wild-type (wt) beta-secretase activity found in most AD patients. Knockout of the CatB gene reduced pGlu-Abeta(3-40/42), flAbeta(1-40/42), and pGlu-Abeta/Abeta plaque load; conversely, expression of CatB increased these Abeta-related peptides. CatB KO reduced CTFbeta derived from APP by beta-secretase. Notably, CatB KO resulted in improved memory deficits assessed by the Morris water maze test. But BACE1 gene KO had no effect on these parameters in the APPLon transgenic mice. These data illustrate the role of CatB in producing pGlu-Abeta and flAbeta that participate as key factors in the development of AD.

Disclosures: **V.Y. Hook:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); equity, American Life Science Pharmaceuticals. **J. Yu:** None. **T. Toneff:** None. **S. Jacobsen:** A. Employment/Salary (full or part-time):; employment, AstraZeneca. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); equity, American Life Science Pharmaceuticals. **M. Kindy:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); equity, Applied Neurological Testing. **G. Hook:** A. Employment/Salary (full or part-time):; employment, American Life Science Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); equity, American Life Science Pharmaceuticals.

Nanosymposium

482. Aggregation of Amyloid, Tau, and Other Proteins

Location: 152A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 482.11

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

Support: DZNE

MPG

WT/MRC

Tau Consortium

Title: Low molecular weight oligomers of Tau repeat domain (TauRD-ΔK280) cause increase in intracellular ROS and calcium levels accompanied by spine loss

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Abstract: The repeat domain of Tau protein with the pro-aggregant mutation ΔK280 (TauRD-ΔK280) induces toxicity in transgenic mice and organotypic hippocampal slice culture models (Sydow A et al., JN 2011, Messing L et al., NBA 2013). One current concept of Tau-mediated toxicity is that it is based on low-n oligomeric species, rather than higher aggregated forms. To test this we characterized oligomers from TauRD-ΔK280 protein assembled and purified in vitro. Since the TauRD-ΔK280 oligomers are not SDS stable, we stabilized them using low concentration of glutaraldehyde as a cross-linking reagent. This yielded SDS stable low molecular weight oligomers predominantly in the form of dimers, trimers, tetramers, with very low amounts of higher order species. The cross-linked TauRD-ΔK280 oligomers can be purified by hydrophobic interaction chromatography with ~95% purity. They exhibit enhanced fluorescence with the dye ANS, arguing for an altered conformation (compared with monomers) and possibly exposed hydrophobic surface patches. However, they do not contain substantial ß-sheet structure, as analyzed by thioflavin S fluorescence and circular dichroism. Atomic force microscopy (AFM) of TauRD-ΔK280 oligomers reveals that the particles are roughly globular in shape, with diameters in the range 1.6-5.4 nm (AFM height values). The hydrodynamic radius of TauRDΔK280 oligomers (~5.2 nm) is dominated by that of tetramers, as measured by dynamic light scattering. The size of TauRD-ΔK280 oligomers from these methods reveal that they contain up to 4-5 molecules of Tau, consistent with the SDS gel analysis. The TauRD-ΔK280 oligomers do not exhibit global toxicity towards rat primary neurons when applied to the extracellular medium, as judged by MTT and LDH assays. However, functional impairment can be deduced from a pronounced (up to 50%) decrease of dendritic spines and a shift of the shape to stubby spines. The neurons also showed an increase in reactive oxygen species and influx of calcium. In summary, low-n oligomers of TauRD-ΔK280 do not cause gross changes in viability, but induce subtle functional defects, leading to increase in Ca++ and ROS, and consequently to loss and shape changes of spines.

Disclosures: S. Kaniyappan: None. **R. Chandupatla:** None. **K. Tepper:** None. **J. Biernat:** None. **E. Mandelkow:** None. **E. Mandelkow:** None.

Nanosymposium

482. Aggregation of Amyloid, Tau, and Other Proteins

Location: 152A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 482.12

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

Support: Alz Assn IIRG 10-173180

NIH AG046200

Title: Methionine restriction reduces Amyloid beta protein accumulation in APP-PS1 transgenic mice

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Abstract: Purpose: The amyloid beta protein (Aβ) accumulates in the brain of Alzheimer's disease patients and is linked to the associated neurodegeneration by a large body of genetic, toxicity and animal model based studies. However, the efforts to treat the disease by removal of amyloid have largely failed. We believe that removal of Aβ at the late stages when dementia becomes manifested will not work as the neuronal dysfunction that takes place several years after Aβ accumulation, presumably due to neuronal death that cannot be reversed. For treatment, we need to identify modifiable risk factors that foster Aβ accumulation and use it for life style changes. Here, we have explored methionine restriction as one such mechanism. Methods: In the first study, cultured cells transfected with human Aβ precursor (APP), were restricted for methionine and media and cell lysates were evaluated for its metabolites. Secondly, we fed transgenic mice expressing human APP a calorie and nitrogen-matched control and methionine restricted diet (75%) for six months and analyzed brain Aβ changes. Results: Although methionine restriction should limit protein synthesis, it did not result in a large reduction of membrane bound full-length APP as it apparently reduced its turnover rate. By contrast the carboxyl-terminal fragment generated by α-secretase cleavage of the precursor (CTFα) drastically dropped in levels. Secreted Aβ was also reduced. Addition of a γ-secretase inhibitor, significantly increased CTFα levels as expected. The relative drop in levels of CTFα in restricted cells was attenuated by γ-secretase inhibition, that reduced α-secretase processing and simultaneously increased γ-secretase processing was responsible for reduced CTFα yield. We therefore predicted that such a restriction would be protective against Aβ accumulation in vivo without causing the simultaneous toxic accumulation of APP CTFs as seen with γ-secretase inhibitors. Methionine restricted mice showed similar weight gain and generally improved health

than control mice as determined by their lower attrition rate, consistent with previous reports on increases in life span by this treatment. Furthermore, soluble Aβ accumulation detected by western blotting and by specific ELISA assays showed significant reductions in the brains of methionine restricted mice. Conclusions: These studies provided evidence that methionine restriction protects APP-transgenic mice against Aβ accumulation. The studies also demonstrate that amino acid restriction can drastically reduce proteolytic processing and turnover of APP by mechanisms that need to be explored further.

Disclosures: K. Sambamurti: None. **M.A. Pappolla:** None. **E. Carley (Hollings):** None. **R.J. Baranello:** None. **V. Padmaraju:** None. **D.K. Lahiri:** None. **N.H. Greig:** None.

Nanosymposium

482. Aggregation of Amyloid, Tau, and Other Proteins

Location: 152A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 482.13

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

Support: National Institute for Translational Neuroscience (INNT/Brazil)

CNPq/Brazil

FAPERJ/Brazil

Human Frontiers Science Program

Canadian Institutes for Health Research

Canada Research Chair Program

Title: PKR-dependent stress signaling underlies memory impairment caused by Alzheimer's disease-linked amyloid-β oligomers

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Abstract: Alzheimer's disease (AD) and type 2 diabetes appear to share similar mechanisms of pathogenesis, including inflammation and metabolic stress. dsRNA-dependent protein kinase (PKR) was recently described as a mediator of peripheral insulin resistance. PKR also phosphorylates eukaryotic translation initiation factor 2α (eIF2α-P) to attenuate protein synthesis, and AD brains exhibit elevated phospho-PKR and eIF2α-P levels. We here describe that amyloid-β oligomers (AβOs), proximal neurotoxins in AD, activate neuronal PKR in a tumor necrosis factor α (TNF-α)- dependent manner, to trigger endoplasmic reticulum (ER) stress, eIF2α-P, synapse loss and memory impairment. Further, we observed that elevated ER stress or eIF2α-P is sufficient to promote cognitive damage in mice. Bolstering brain insulin signaling with anti-diabetic agents rescued phospho-PKR and eIF2α-P caused by AβOs. Results shed light on novel mechanisms shared by AD and diabetes that might account for impaired cognition. These observations provide novel targets for drug development in AD and offer new information on how anti-diabetic agents protect the brain from AD-related insults.

Disclosures: **M.V. Lourenço:** None. **J.R. Clarke:** None. **R.L. Frozza:** None. **T.R. Bomfim:** None. **L. Forny-Germano:** None. **A.F. Batista:** None. **L.B. Sathler:** None. **J. Brito-Moreira:** None. **O.B. Amaral:** None. **L. Freitas-Correa:** None. **J. Houzel:** None. **W.L. Klein:** None. **C. Holscher:** None. **J.B. Carvalheira:** None. **A.M. Silva:** None. **L.A. Velloso:** None. **D.P. Munoz:** None. **S.T. Ferreira:** None. **F.G. De Felice:** None. **C.A. Silva:** None. **S. Espírito-Santo:** None. **P. Campello-Costa:** None.

Nanosymposium

483. Modeling APP and Abeta Pathology in Animals

Location: 147B

Time: Tuesday, November 18, 2014, 8:00 AM - 10:15 AM

Presentation Number: 483.01

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

Support: ISG 5 P30 AG013319-18 NIH

OWENS FND William & Ella Owens Medical Research Foundation

RC2AG036613 NIH

JMR BARKER FND Barker Foundation

Title: TOR as a key regulator of brain vascular function in mouse models of Alzheimer's disease

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Abstract: We recently showed that chronic treatment with the TOR inhibitor rapamycin, a drug that extends lifespan and delays aging in mice, halted and even rescued progression of Alzheimer's (AD)-like memory deficits and reduced accumulation of Aβ in hAPP(J20) mice modeling the disease. Chronically reducing TOR activity with rapamycin after the onset of AD-like symptoms negated profound reductions in cerebral blood flow (CBF) and vascular density that were not related to metabolic changes in hAPP(J20) mice, especially in brain areas with a prominent role in learning and memory, via eNOS activation in brain vascular endothelial cells. Reduction of TOR activity also restored CBF and learning in aged rats, as well as in a mouse model of atherosclerosis, suggesting that the mechanisms by which attenuation of mTOR activity restores CBF and cognitive competence are common to different models of age-associated neurological disease, and to brain aging. Because endothelial function declines with age, and loss of blood-brain barrier (BBB) integrity resulting from Aβ toxicity has been implicated in AD pathogenesis, we used in vivo multiphoton imaging to monitor changes in BBB integrity associated with AD-like histopathology and AD-like progression in hAPP(J20) mice and to determine dynamics of vascular Aβ deposition in brains of Tg2576 mice, that develop robust AD-associated vascular deficits including cerebral amyloid angiopathy (CAA). Our results demonstrate that chronically reducing TOR activity abrogated severe BBB damage and abolished memory impairments at very late stages of AD-like disease in hAPP(J20) animals and pronouncedly reduced Aβ pathology in Tg2576 mice. TOR inhibition was linked to restoration of BBB integrity through the upregulation of tight junction protein expression in brain vascular endothelial cells. Our data suggest that chronic TOR attenuation precludes AD-like vascular damage in two independent models and prevents progression of AD-like cognitive deficits through late stages of AD-like disease in mice. We propose that, in addition to the activation of eNOS, TOR regulates the maintenance of brain vascular function through the maintenance of BBB integrity during the progression of AD-like disease, reducing Aβ accumulation. Rapamycin, an FDA-approved drug that is already used in the clinic, or other TOR inhibitors, may have promise as therapy for AD and possibly for age-associated brain diseases beyond AD alone.

Disclosures: **V. Galvan:** None. **A. Lin:** None. **S. Hussong:** None. **N. Sayre:** None. **J. Halloran:** None. **R. Burbank:** None. **S. Austad:** None. **K. Fischer:** None. **J.D. Lechleiter:** None. **R. Asmis:** None.

Nanosymposium

483. Modeling APP and Abeta Pathology in Animals

Location: 147B

Time: Tuesday, November 18, 2014, 8:00 AM - 10:15 AM

Presentation Number: 483.02

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

Support: Fritz Thyssen Foundation (TEW)

Alzheimer Research Initiative/AFI (TEW)

Humboldt Postdoctoral Fellowship (SBD)

Title: The relevance of retromer- vs. GGA-dependent transport of SORLA for amyloidogenic processes *in vivo*

Authors: ***S. B. DUMANIS**, T. BURGERT, S. CAGLAYAN, V. SCHMIDT, T. E. WILLNOW;
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Abstract: SORLA/SORL1 is a neuronal sorting receptor implicated in both familial and sporadic forms of Alzheimer's disease (AD). According to the current hypothesis, SORLA has two distinct functions in control of amyloidogenic processes. Specifically, SORLA can impact processing by sorting internalized APP molecules from endosomes back to the trans-Golgi network (TGN) reducing amyloidogenic processing of the precursor protein in endosomal compartments. Additionally, SORLA can impact clearance by directing newly produced Ab; to lysosomes for intracellular catabolism, further decreasing amyloidogenic burden. **G**olgi-localizing **g**amma-adaptin ear homology domain **A**RF-interaction proteins (GGAs) and the retromer complex are cytosolic adaptors known to interact with the cytoplasmic domain of SORLA, thereby controlling intracellular routing of the receptor in established cell lines. Specifically, GGA is associated with anterograde, and retromer is associated with retrograde transport of the receptor. Here, we examine the biological relevance of these adaptor-mediated sorting pathways of SORLA for receptor function *in vivo*. To do so, we generated new mouse models which lack endogenous SORLA (*Sorl1*$^{-/-}$) but carry transgenes under the control of the

Rosa26 promoter encoding for wild-type SORLA or trafficking mutants of the receptor in which binding sites for GGA or retromer have been disrupted; and backcrossed these animal models to an AD mouse model (5xFAD). We found that the disruption of the SORLA and retromer interaction results in the inability of SORLA to sort APP from endosomes back to the TGN in neurons. As a consequence of endosomal accumulation, APP is subject to excessive processing as evidenced by an overall increase in brain levels of soluble (s) APPb and Ab in receptor mutant mice. In contrast, the disruption of the SORLA and GGA interaction did not alter the levels of sAPPb but did increase Ab levels *in vivo*, a mechanism attributed to a defect in lysosomal targeting of Ab. Collectively, our findings provide experimental proof for the significance of adaptor-mediated sorting of SORLA for amyloidogenic processes *in vivo*, and they suggest distinct functions for retromer and GGA in SORLA-dependent sorting of APP and Ab, respectively.

Disclosures: S.B. Dumanis: None. **T. Burgert:** None. **S. Caglayan:** None. **V. Schmidt:** None. **T.E. Willnow:** None.

Nanosymposium

483. Modeling APP and Abeta Pathology in Animals

Location: 147B

Time: Tuesday, November 18, 2014, 8:00 AM - 10:15 AM

Presentation Number: 483.03

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

Support: CIHR grant MOP102752

Title: Proteomic analyses reveal altered hippocampal protein expression in a rat model of Alzheimer’s disease

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Abstract: The cerebral accumulation and cytotoxicity of amyloid beta peptides is the main cause of Alzheimer’s disease (AD). However, we do not know how this amyloidogenic pathology affects the global CNS expression of proteins which might contribute to the disease progression. Towards this objective, we used the McGill-R-Thy1-APP rat model of AD-like amyloid pathology. Because of their complex CNS organization, physiology and superior cognitive abilities, rat models are closer to the human condition as compared to mice. McGill-APP

transgenic rats were compared to wild-type rats at two time-points: at three months of age when the amyloid pathology is only present in the intracellular compartment but cognitive deficits are already present; and at twelve months of age when extracellular plaques are abundantly present and the cognitive deficits have progressed. Quantitative proteomics analyses of hippocampal tissue were carried out using isobaric tagging for relative and absolute quantitation (iTRAQ) with liquid chromatography/mass spectrometry analyses in a Q-Exactive Orbitrap to identify genotype and time-dependent changes in protein expression. After correction for multiple testing, expression levels of 64 proteins were found to be considerably different in transgenic versus control rats at 3 months of age, and 86 proteins in the 12 month age group. Datasets of significantly regulated proteins were uploaded to Ingenuity Pathway Analysis software for interpretation of the functional implication of the observed changes. Interestingly, the proteins affected at the pre-plaque stage (3 months) vary widely from the proteins impacted at the post-plaque stage (12 months), with only 9 proteins common to the two time-points. This minimal overlap supports the hypothesis that distinctive processes are at work in the hippocampus during the progression of AD. Oxidative stress processes are prominent at early stages while disturbances in amino acid metabolism develop later on. Further exploration of these changing molecular profiles could lead to identification of novel therapeutic targets for early intervention in AD.

Disclosures: S. Do Carmo: None. **G. Crynen:** None. **A. Ducatenzeiler:** None. **F. Crawford:** None. **A. Cuello:** None.

Nanosymposium

483. Modeling APP and Abeta Pathology in Animals

Location: 147B

Time: Tuesday, November 18, 2014, 8:00 AM - 10:15 AM

Presentation Number: 483.04

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

Support: UAB Faculty Development Grant

NEI P30 EYE3039

Title: Changes in the retinal cholinergic system and retinal cell loss as potential contributors to visual deficits in Tg-SwDI and J20 mouse models of Alzheimer’s disease

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Abstract: Alzheimer's disease (AD) is characterized by severe cognitive deficits as well as by visual deficits in motion perception, contrast sensitivity, acuity and color. To assess the potential contributions of the retinal cholinergic system to these visual deficits we assessed changes in acetylcholine receptor (AChR) transcripts expression and retinal cell loss in the Tg-SwDI and J20 mouse models of AD. Tg-SwDI mice express the human amyloid precursor protein (hAPP) with the Swedish KM670/671NL, Dutch E693Q, and Iowa D694N mutations. J20 mice overexpress hAPP with the Swedish and the Indiana V717F mutations. We conducted quantitative real-time polymerase chain reaction (qPCR) with validated and optimized primers using whole-retina RNA. We observed statistically significant ($\leq$0.05) differences in nicotinic (nAchR) and muscarinic (mAchR) mRNA transcripts in the retinas of AD mice as compared to age-matched wild type (WT) mice. J20 mice (6-9 months old (mo)) exhibited 5-8 fold upregulation of nAchR alpha subunit transcripts 2, 4,7and 9. Tg-SwDI retinas (9-12 mo) showed much higher (4-20 fold) upregulation of nAchR subunits (alpha 2, 3, 4, 6, 9 & 10; Beta 3 & 4) and mAChR subtype (m1&m5) transcripts, as well as a 0.15 fold downregulation in nAchR alpha 5 subunit transcripts. The upregulation of nAchR alpha 2, 4, 7 and 9 subunits, and mAchR m5 subtype transcripts was smaller (0.1-0.22 fold) in the retinas of older Tg-SwDI (12-15 mo) while the other nAChR transcripts were no longer upregulated. Immunohistochemistry (IHC) was performed in whole-mount retinas using antibodies against choline acetyltransferase (ChAT). ChAT immunoreactive (IR) and Hoescht-labeled cell nuclei were counted in images obtained from 8 regions of interest per retina in the ganglion cell, inner, and outer nuclear layers (GCL, INL and ONL). Tg-SwDI retinas had fewer total cells in the INL and GCL at 9 mo as compared to age-matched WT mice ($p<0.001$). ChAT-IR in the GCL ($p<0.001$), but not the INL, was also reduced at 9 mo. Reductions in the number of ONL cells were apparent by 15 mo ($p<0.001$), as were further reductions in the number of ChAT-IR cells in the INL ($p<0.001$) and in the GCL ($p<0.001$).Thus, cell loss occurs earliest in the inner retinas of Tg-SwDI mice and extends through the outer retinas by 14 mo. The early upregulation of AChR transcripts might compensate, in part, for the loss of cholinergic cells. Understanding the causes of the visual deficits may be crucial in early AD diagnosis, as they may occur before cognitive decline is observed. This work is an important step in the quest towards attaining a better understanding of AD and facilitating its early diagnosis and treatment.

Disclosures: F.G. Olivera Souza: None. **C.E. Strang:** None. **T. van Groen:** None. **E.D. Roberson:** None. **K.T. Keyser:** None.

Nanosymposium

483. Modeling APP and Abeta Pathology in Animals

Location: 147B

Time: Tuesday, November 18, 2014, 8:00 AM - 10:15 AM

Presentation Number: 483.05

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

Support: NIH Grant RO1 N5047225

NIH Grant P50 AG005146

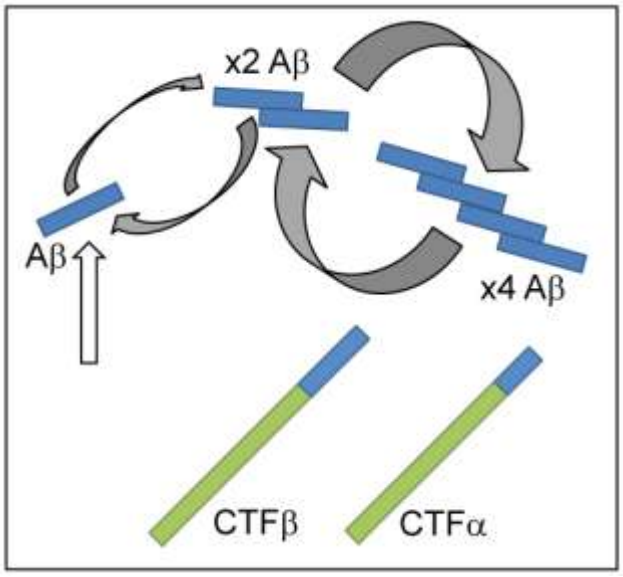
Supplement for Sex-related differneces to NIH Grant P50 AG005146

Title: Conditional CaMKIItTa:APPsi models of Alzheimer's disease have similar APP expression in males and females and highly correlated levels of Aβ dimers and tetramers

Authors: *A. V. SAVONENKO[1], T. MELNIKOVA[1], L. BECKER[1], E. CHO[1], D. LEE[1], N. SAYYIDA[1], J. TIAN[2], K. J. BANDEEN ROCHE[2], D. BORCHELT[3];
[1]Pathology, Johns Hopkins Univ., Baltimore, MD; [2]Sch. of Publ. Hlth., The Johns Hopkins Univ., Baltimore, MD; [3]McKnight Brain Inst., Univ. of Florida, Gainesville, FL

Abstract: Transgenic models of Alzheimer's disease (AD) advance our understanding of the pathogenesis of this disease and are instrumental in discoveries and validation of therapeutic targets. In this study, we used a mouse model of Alzheimer-type amyloidosis in which the deposition of Aβ is driven by the expression of mutant amyloid precursor protein (APP) under the transcriptional control of a tetracycline regulated promoter. This model utilizes a bigenic approach in which expression of a chimeric mouse/human APP with the Swedish and Indiana mutations is driven by co-expression of the tetracycline transactivator (tTA) under the transcriptional control of the CaMKIIα promoter. In contrast to APP transgenic models in which overexpression of APP is driven by Prion promoter, CaMKIItTa:APPsi models result in levels of APP expression that is not significantly affected by sex-related factors as observed in two different lines (Lines 107 and 885). APP processing by β- and α-secretases as well as levels of monomeric and oligomeric Aβ species are similar between males and females indicating that CaMKIItTa:APPsi models are appropriate for studying mechanisms of sex-related sensitivity to Aβ. Stepwise multiple regression applied to explain levels of oligomers as observed by Western in RIPA-solubilized forebrain samples (Fig) revealed that tetramers had one significant predictor, dimers, with strength of determination rarely observed in biological systems (92%). In addition, most of the variance of the dimers was uniquely explained by the levels of tetramers (83%). Dimers were present only in SDS-PAGE of brain samples and disappeared after spiking with

Ab42. These data indicate an existence of strong dynamic equilibrium between SDS-stable Aβ dimers and tetramers.



Disclosures: **A.V. Savonenko:** None. **T. Melnikova:** None. **L. Becker:** None. **E. Cho:** None. **D. Lee:** None. **N. Sayyida:** None. **J. Tian:** None. **K.J. Bandeen Roche:** None. **D. Borchelt:** None.

Nanosymposium

483. Modeling APP and Abeta Pathology in Animals

Location: 147B

Time: Tuesday, November 18, 2014, 8:00 AM - 10:15 AM

Presentation Number: 483.06

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

Support: AAS-NIRG-173407

MIUR-Ageing project –"Research on the Molecular Mechanisms Involved in Ageing"

Title: Entorhinal cortex dysfunction is an early sign of neurodegeneration in Alzheimer's disease mouse model

Authors: C. CRISCUOLO[1], S. S. D. YAN[2], *N. ORIGLIA[3];
[1]DISCAB, Univ. of L'Aquila, L'Aquila, Italy; [2]Pharmacol. and Toxicology, Univ. of Kansas, Lawrence, KS; [3]CNR- Neurosci. Inst., PISA, Italy

Abstract: Entorhinal cortex (EC) is a parahippocampal region involved in learning and memory and exhibiting an high degree of plasticity. The EC has been implicated in the early stages of Alzheimer's disease (AD). In particular, cortical-hippocampal network dysfunction in the human amyloid precursor protein (hAPP-j20) mouse model has been reported, suggesting a spreading of neuronal dysfunction within the EC-Hippocampal network. Our aim was to investigate the time course of EC synaptic plasticity impairment in hAPP mice at different stages of neurodegeneration. To this aim, we recorded extracellular field potentials from cortical layer II/III stimulating the horizontal pathway in layer II of in vitro EC slices. Moreover, using an associative-recognition memory task we have evaluated whether synaptic plasticity deficits were associated with a behavioural impairment. We found that long term potentiation (LTP) was specifically impaired in EC slices from 2 month old hAPP mice as long-term depression (LTD), paired pulse facilitation and basic synaptic transmission were not altered and were comparable to wild-type littermate. At this early stage of neurodegeneration the LTP deficit in hAPP slices was restricted to the EC as the expression of LTP in the dentate gyrus (DG) of hippocampus was not affected. With the progression of neurodegeneration (5 to 6 month old mice), EC synaptic dysfunction in hAPP slices also involved LTD expression and basic synaptic transmission. The temporal profile of EC dysfunction was confirmed by behavioural analysis in 2 month old mice using an associative task (object-context recognition) which requires EC integrity. Indeed, after a lesion in the lateral EC, wild-type mice revealed a normal recognition ability in the non-associative task but a selective impairment in the object-context recognition test (OCRT). Similar results were obtained in non-lesioned 2 month old hAPP mice which displayed a selective impairment in the OCRT. These results suggest that early synaptic deficit in the EC may underlie a specific memory impairment in hAPP mice. Our experiments support the crucial involvement of the EC in the development of synaptic and behavioral deficits during AD neurodegeneration.

Disclosures: **C. Criscuolo:** None. **S.S.D. Yan:** None. **N. Origlia:** None.

Nanosymposium

483. Modeling APP and Abeta Pathology in Animals

Location: 147B

Time: Tuesday, November 18, 2014, 8:00 AM - 10:15 AM

Presentation Number: 483.07

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

Support: MR/K022105/1

Title: A novel amyloid beta model: Linking long term memory defects to altered signaling in CREB pathways

Authors: *L. FORD, M. CROSSLEY, J. R. THORPE, L. C. SERPELL, G. KEMENES; Neurosci., Univ. of Sussex, Falmer, United Kingdom

Abstract: The dysfunctioning mechanisms of amyloid β- induced dementia are still elusive, regardless of the advances in Alzheimer's Disease research in recent years. We believe that part of the current issues in amyloid β research include inconsistencies between labs and a lack of ability to link many studies back to behavioral deficits. For this reason, we have utilized an animal model which has been very advantageous to learning and memory research and have expanded its use to amyloid β research. Lymnaea stagnalis offers a tractable model with high evolutionary conservation of key molecular pathways and fundamental functions of learning also present in mammalian models. Importantly, Lymnaea allows us to use a top-down approach in our experiments which has enabled us to link behavioral deficits to cellular, network, and molecular dysfunction with a high level of experimental control between levels of study. We implemented different techniques in order to develop Lymnaea as a useful model for the amyloid β field, including observing behavioral deficits of long term memory, tracking injected amyloid β's entry into brain tissue and neurons, and measuring altered cellular properties of neurons. We then conducted an in-depth analysis of multiple CREB-signaling pathways involved in long term memory using molecular techniques, behavioral pharmacology, and biochemical assays which revealed that amyloid β treatment alters specific key molecular components through down-regulation and decreased protein availability. Linking these pathway abnormalities to neuronal and network properties, as well as behavior, has allowed us to help elucidate the fundamental mechanisms involved in the amyloid β-induced memory dysfunction.

Disclosures: L. Ford: None. **L.C. Serpell:** None. **G. Kemenes:** None. **J.R. Thorpe:** None. **M. Crossley:** None.

Nanosymposium

483. Modeling APP and Abeta Pathology in Animals

Location: 147B

Time: Tuesday, November 18, 2014, 8:00 AM - 10:15 AM

Presentation Number: 483.08

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

Support: DFG grant 1457/9-1, 9-2

KO 1674/3-1, 3-2

BMBF grant 01GS08128

Title: Conditional APP/APLP2 double knockout mice reveal essential functions in excitatory principal neurons for spine density, synaptic plasticity and cognition

Authors: *U. MULLER[1], M. HICK[1], U. HERRMANN[2], D. P. WOLFER[3], M. KORTE[2]; [1]Inst. Pharm. & Mol Biotech IPMB, Heidelberg Univ., Heidelberg, Germany; [2]Zoological Institute, Cell. Neurobio., TU Braunschweig, Braunschweig, Germany; [3]Dept. of Anat., Zurich Univ., Zurich, Switzerland

Abstract: The key role of APP in AD pathogenesis is well established. However, functional compensation within the APP gene family and postnatal lethality of double knockout (KO) mice has so far precluded the analysis of the physiological functions of APP and the APLPs. To circumvent lethality of APP/APLP2 double KO mice and to further elucidate the role of APP and APLP2 in the adult CNS we generated animals with a conditional APP/APLP2 double KO (cDKO) in excitatory forebrain neurons. To this end we crossed APPflox/APLP2KO mice to NEX-Cre mice expressing Cre prenatally in postmitotic neuronal progenitor cells. Western blot analysis, immunohistochemistry and *in situ* hybridization indicated a widespread loss of APP specifically in excitatory forebrain neurons including cortex and hippocampus, whereas interneurons were not affected. Histological analysis revealed no gross alterations in hippocampal or cortical morphology and no apparent activation of astrocytes as unspecific signs of brain damage was detectable. Previously, APP family proteins have been implicated in synaptic adhesion and analysis of the neuromuscular junction of APP/APLP2 mutant mice showed pronounced deficits in synaptic morphology and impaired neuromuscular transmission (Weyer et al., 2011). We therefore assessed dendritic spine density of hippocampal CA1 neurons in young adult (4-5 months old) mice by iontophoretic neuronal filling with a fluorescent dye. Quantitative analysis revealed a significantly reduced spine density in both apical and basal dendrites of NEX-Cre cDKO mice as compared to littermate controls. Electrophysiological recordings of NEX-Cre cDKOs indicated a strong synaptic phenotype with pronounced deficits in the induction and maintenance of hippocampal LTP. In addition, young adult NEX-Cre cDKOs showed impairments in paired pulse facilitation, indicating a possible presynaptic deficit. Synaptic deficits were also associated with impairments in nesting behavior and hippocampus dependent learning and memory tasks, including deficits in Morris water maze and radial maze performance. Collectively, our analysis reveals an essential and combined role of APP and APLP2 in excitatory principal neurons for mediating normal spine density, synaptic plasticity and cognition.

Disclosures: U. Muller: None. **M. Hick:** None. **U. Herrmann:** None. **D.P. Wolfer:** None. **M. Korte:** None.

Nanosymposium

483. Modeling APP and Abeta Pathology in Animals

Location: 147B

Time: Tuesday, November 18, 2014, 8:00 AM - 10:15 AM

Presentation Number: 483.09

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

Support: Engebretson/Bighley Drug Design and Development Program, College of Pharmacy, University of Minnesota

Title: Therapeutic potential of a novel neuroprotective compound - TH-237A for Alzheimer's disease

Authors: *D. A. HOTTMAN[1], Z. WANG[1], G. I. GEORG[2], L. LI[1];
[1]Exptl. and Clin. Pharmacol., [2]Medicinal Chem., Univ. of Minnesota, Minneapolis, MN

Abstract: Alzheimer's Disease (AD) is a devastating degenerative neurological disease that affects nearly 5.5 million Americans. The incidence of AD is expected to grow as the population of the United States ages. Clinically, AD is characterized by progressive cognitive impairments. Pathologically, AD is defined by amyloid-β (Aβ) plaques and neurofibrillary tau tangles. Multiple lines of evidence indicate that Aβ and tau play synergistic roles in the development of AD. Several studies suggest that Aβ-induced neuritic dystrophy and loss of cytoskeletal integrity may result from the loss of microtubule stabilization by tau. Indeed, several microtubule-stabilizing agents such as paclitaxel (Taxol®) and epothilone D protect cultured neurons from the toxic effects of Aβ. In the search for a microtubule-interacting compound that possesses not only the potent neuroprotective property but also the ability to reach the site of action within the brain, a unique small molecule derived from paclitaxel, designated as TH-237A, was identified. TH-237A exhibits low nanomolar potency in protecting cultured neurons against Aβ and possesses the properties to penetrate the blood brain barrier. The present study was designed to investigate the potential of TH-237A for mitigating AD-like neuropathology in vivo.TH-237A was administered (10mg/kg body weight) daily to 6 to 10-month old APPsw/PS1dE9 mice, an established model of AD, by subcutaneous injections for 4 months. Vehicle-treated littermates were used as controls. There was no difference in body weight gain between TH-237A and vehicle treated mice, suggesting little toxicity from the chronic daily treatment. Immunohistochemical analysis showed that the Aβ load was reduced significantly in mice treated with TH-237A. Additionally, the immunoreactivity of IBA-1, a commonly used marker for microglia activation, was also significantly attenuated in TH-237A treated mice. Further studies are underway to determine the mechanism(s) underlying the beneficial effects of TH-237A. These exciting results demonstrate the therapeutic potential of TH-237A for AD.

Disclosures: **D.A. Hottman:** None. **Z. Wang:** None. **G.I. Georg:** None. **L. Li:** None.

Nanosymposium

484. Alpha-Synuclein and LRRK2 Mechanisms in Parkinson's Disease

Location: 146C

Time: Tuesday, November 18, 2014, 8:00 AM - 10:45 AM

Presentation Number: 484.01

Topic: C.03. Parkinson's Disease

Title: 4-Hydroxynonenal triggers proteopathic alterations in alpha-synuclein metabolism: Potential role in Parkinson's disease

Authors: ***S. ZHANG**, E. EITAN, M. P. MATTSON;
NIH, Baltimore, MD

Abstract: α -synuclein (α-syn) inclusions in degenerating neurons in the brainstem, substantia nigra and some regions of cerebral cortex are pathological hallmarks of Parkinson's disease (PD). The mechanisms underlying the accumulation and spreading of aberrant α-syn aggregates is largely unknown. 4-hydroxynonenal (HNE), a lipid peroxidation product generated in response to oxidative stress, increases in neurons during normal aging and more so in vulnerable neurons in PD. In our study, we found that HNE can induce neurodegeneration in rat cerebral cortical neurons in a dose-dependent and time-dependent pattern. Levels of α-Syn oligomers (high molecular weight α-Syn) were increased in HNE-treated neurons compared with vehicle-treated neurons in both 1% NP-40 soluble and insoluble cellular fractions. In addition to accumulation of α-Syn oligomers inside neurons, HNE-treated neurons secreted more α-Syn oligomers that were contained within exosomes. Furthermore, exosomes from HNE-treated neurons were internalized by naïve neurons resulting in the intracellular accumulation of Thioflavin S-reactive aggregates. We are currently working to determine the molecular mechanism by which HNE causes exosome-mediated release of pathogenic forms of α-syn. In conclusion, our findings suggest that HNE, the levels of which are elevated in the brains of PD patients, may play roles in the intracellular accumulation and trans-neuronal propagation of α-syn pathology.

Disclosures: **S. Zhang:** None. **E. Eitan:** None. **M.P. Mattson:** None.

Nanosymposium

484. Alpha-Synuclein and LRRK2 Mechanisms in Parkinson's Disease

Location: 146C

Time: Tuesday, November 18, 2014, 8:00 AM - 10:45 AM

Presentation Number: 484.02

Topic: C.03. Parkinson’s Disease

Support: NIH Grant NS067024

Title: Rab protein effects on alpha-synuclein homeostasis and oligomer formation

Authors: *N. R. MCFARLAND, T. SAHARA, M. HERRING, H.-J. PARK, D. RYU, R. COULTAS;
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Abstract: In addition to the progressive loss of dopaminergic neurons, a major pathological hallmark of Parkinson disease is the presence of Lewy bodies that are enriched with the protein, alpha-synuclein (αS). Abnormal accumulation and deposition of αS are associated with ER stress, intracellular vesicle trafficking deficits, and cytotoxicity (Cooper et al, Science 2007). Vesicle trafficking deficits and toxicity caused by αS overexpression can be mitigated by expression of specific Rab proteins (Gitler et al, PNAS 2008), suggesting that abnormal accumulation of αS may interfere with normal Rab protein functions. Additionally, RabGTPases may play important role in αS homeostasis and reduce formation of toxic oligomers and aggregate formation. In this study, we examine the effects of specific Rab proteins, including mouse Rab1, its human paralog Rab8A, and Rab 3A, on αS-dependent toxicity and oligomer formation in neuronal cell models. Using a protein complementation assay which utilizes αS constructs fused to N and C-terminal fragments of the Gaussian luciferase molecule or Venus-YFP (Outiero et al, PLoS One 2008), we are able to quantify αS dimer/oligomer formation. Co-expression of mRab1 and Rab8A with αS-luciferase N and C-terminal fragments in H4 neuroglioma cells significantly reduced luciferase activity compared to control, indicating reduction in dimer/oligomer species. In contrast Rab3A had no apparent effect on αS dimer/oligomer formation. Western blot and size-exclusion data further support a reduction not only in high-molecular weight αS species, but also total αS. Rab8A in particular showed the greatest reduction in αS levels, which also correlated with reduced Golgi fragmentation in cells. Molecular studies indicate a post-translational mechanism for Rab8A effects on αS, but these appear to involve degradation independent of the UPS or macroautophagy system. Further studies are necessary to elucidate the mechanism of Rab8A effects on αS levels and whether Rab8A expression protects against αS-toxicity in Parkinsons.

Disclosures: **N.R. McFarland:** None. **T. Sahara:** None. **M. Herring:** None. **H. Park:** None. **D. Ryu:** None. **R. Coultas:** None.

Nanosymposium

484. Alpha-Synuclein and LRRK2 Mechanisms in Parkinson's Disease

Location: 146C

Time: Tuesday, November 18, 2014, 8:00 AM - 10:45 AM

Presentation Number: 484.03

Topic: C.03. Parkinson's Disease

Support: NIH K08 NS067024

Title: Retention in endoplasmic reticulum (Rer1) promotes alpha-synuclein degradation

Authors: ***H.-J. PARK**[1], D. RYU[1], L. RICCHIUTI[1], B. GIASSON[2], N. MCFARLAND[1]; [1]CTRND/Department of Neurol., [2]CTRND/Department of Neurosci., Univ. of Florida, Gainesville, FL

Abstract: Alpha-synuclein (αSyn) is a principle component of Lewy bodies found pathologically in Parkinson disease (PD) and related "synucleinopathies." Numerous studies suggest that accumulation, aggregation, and deposition of αSyn correlate with the development of neuronal dysfunction and degeneration. Our work has focused on finding cellular factor(s) regulating αSyn degradation/accumulation for future therapeutic approaches. Several recent studies have linked αSyn accumulation and toxicity to defective intracellular protein/vesicular trafficking and impairment of endoplasmic reticulum-associated degradation (ERAD), suggesting that modulation of protein transport and homeostasis could ameliorate αSyn toxicity. Rer1 has been identified as an ER retrieval/retention factor for Alzheimer's disease (AD) proteins which negatively regulates Amyloid beta peptides, the key pathogenic player in AD (Park HJ, et al. JBC 2012). In our study, the levels of αSyn wild type, A30P, A53T, or E46K mutant were assessed following Rer1 co-expression in human cell lines. Rer1 significantly decreased both αSyn wild type and mutants levels. However, the Rer1Δ25 mutant, which lacks the C-terminus that appears important for substrate localization, had a significantly attenuated effect on αSyn, indicating that Rer1-mediated ER recycling may be important for regulating αSyn levels. Interestingly, proteasome inhibition rescues the Rer1-mediated decrease in αSyn levels suggesting that Rer1 facilitates αSyn degradation through ubiquitin-proteasome system (UPS) and possibly through ERAD. Immunohistochemistry and immunofluorescence assays using human tissues further show that Rer1 colocalizes with αSyn in Lewy bodies. These data addresses the mechanism of a potential novel mediator of αSyn levels, Rer1, which may play a

critical role in protein trafficking and regulation of αSyn and associated toxicity in Parkinson disease and related disorders.

Disclosures: H. Park: None. **D. Ryu:** None. **L. Ricchiuti:** None. **B. Giasson:** None. **N. McFarland:** None.

Nanosymposium

484. Alpha-Synuclein and LRRK2 Mechanisms in Parkinson's Disease

Location: 146C

Time: Tuesday, November 18, 2014, 8:00 AM - 10:45 AM

Presentation Number: 484.04

Topic: C.03. Parkinson's Disease

Support: DFG Center for Nanoscale Microscopy and Molecular Physiology of the Brain

Title: Glycation disrupts proteostasis and promotes neurodegenerative alterations in synucleinopathies

Authors: *T. F. OUTEIRO[1], H. VICENTE-MIRANDA[2], E. M. SZEGO[1], W. XIANG[3], H. LASHUEL[4], J. KLUCKEN[5], M. ZWECKSTETTER[6], L. V. LOPES[2], F. GIORGINI[7]; [1]Dept. of Neurodegeneration and Restorative Res., Univ. Med. Ctr. Goettingen, Goettingen, Germany; [2]Inst. de Medicina Mol., Lisbon, Portugal; [3]Friedrich-Alexander-University Erlangen-Nürnberg, Nuernberg, Germany; [4]EPFL, Lausanne, Switzerland; [5]Univ. Hosp. Erlangen, Erlangen, Germany; [6]DZNE, Goettingen, Germany; [7]Univ. of Leicester, Leicester, United Kingdom

Abstract: α-synuclein (aSyn) aggregation in Lewy bodies is a pathological hallmark of Parkinson's disease (PD) and other synucleinopathies. Glycation, an age-dependent protein modification, is present in Lewy bodies. Here, we investigated the effect of the natural glycating agent methylglyoxal on aSyn biology and found that glycation increased aSyn aggregation and toxicity. Notably, striatal injection of methylglyoxal in mice caused neuronal loss. Genetic and pharmacological manipulation of methylglyoxal increased aSyn dependent toxicity in human LUHMES cells and in PD patient-derived iPSCs, and decreased motor performance and survival in aSyn transgenic flies. Furthermore, glycated aSyn impaired synaptic transmission in rat hippocampal slices. Methylglyoxal promoted aSyn oligomerization by affecting its N-terminal structure and impairing lipid-binding ability. Glycation disrupted proteostasis, reducing aSyn turnover, aggregation, and release, likely the mechanistic link underlying the phenotypes

observed. In total, our study uncovers glycation as a novel player in synucleinopathies, opening novel avenues for the design of therapeutic strategies.

Disclosures: **T.F. Outeiro:** None. **E.M. Szego:** None. **H. Vicente-Miranda:** None. **F. Giorgini:** None. **J. Klucken:** None. **H. Lashuel:** None. **M. Zweckstetter:** None. **L.V. Lopes:** None. **W. Xiang:** None.

Nanosymposium

484. Alpha-Synuclein and LRRK2 Mechanisms in Parkinson's Disease

Location: 146C

Time: Tuesday, November 18, 2014, 8:00 AM - 10:45 AM

Presentation Number: 484.05

Topic: C.03. Parkinson's Disease

Title: Characterization of surface-exposed epitopes of *in vitro* and *in vivo* formed alpha-synuclein aggregates

Authors: *J. BERGSTROM[1], L. ALMANDOZ-GIL[1], J. SIGVARSSSON[2], L. LANNFELT[1], M. INGELSSON[1];
[1]Uppsala Univ., Uppsala, Sweden; [2]BioArctic Neurosci., Stockholm, Sweden

Abstract: Background: Alpha-synuclein is the main component of the intraneuronal Lewy body inclusions found in disorders such as Parkinson's disease and dementia with Lewy bodies. Alpha-synuclein consists of an amphipathic amino-terminal region (1-60), a hydrophobic mid region (61-95) and a negatively charged hydrophilic carboxyl-terminus (96-140). Under certain circumstances alpha-synuclein monomers undergo conformational changes, forming oligomers of increasing sizes, and eventually insoluble fibrils. However, it is not known exactly which parts of the molecule that are exposed in the inclusions. **Objective:** To determine surface exposed epitopes of alpha-synuclein aggregates *in vitro* and *in vivo*. **Methods:** Polyclonal chicken antibodies were raised against short linear epitopes spanning the entire molecule. Monomeric alpha-synuclein was expressed recombinantly and oligomers and fibrils were generated. Brain tissue from transgenic (tg*) A30P* alpha-synuclein mice, and from patients with Parkinson's disease and dementia with Lewy bodies were available for immunohistochemical and biochemical analyses. **Results:** The surface exposed epitopes of *in vitro* generated monomeric, oligomeric and fibrillar alpha-synuclein were assessed. Apart from C-terminal epitopes, both oligomeric and fibrillar alpha-synuclein were found to have two N-terminal epitopes exposed. Antisera against these two N-terminal epitopes also labeled neuropil threads and neuronal cell bodies in pre-symptomatic tg brain and Lewy bodies in diseased human brain tissue. Ongoing

studies will further characterize these N-terminal epitopes. **Conclusions:** The exact structure of *in vitro* and *in vivo* formed aggregates has not yet been determined. By the use of sequence-specific polyclonal antibodies, surface exposed epitopes of various alpha-synuclein species can be identified. Such knowledge can be important for future development of diagnostic and therapeutic approaches for patients with Lewy body disorders.

Disclosures: J. Bergstrom: None. **L. Almandoz-Gil:** None. **J. Sigvarssson:** A. Employment/Salary (full or part-time):; BioArctic Neuroscience. **L. Lannfelt:** None. **M. Ingelsson:** None.

Nanosymposium

484. Alpha-Synuclein and LRRK2 Mechanisms in Parkinson's Disease

Location: 146C

Time: Tuesday, November 18, 2014, 8:00 AM - 10:45 AM

Presentation Number: 484.06

Topic: C.03. Parkinson’s Disease

Support: Cure Parkinson's Trust Project Grant

Parkinson's UK Innovation Grant

Title: Silent pathological oligomeric alpha-synuclein revealed in Parkinson’s disease brain

Authors: *R. ROBERTS, R. WADE-MARTINS, J. ALEGRE-ABARRATEGUI;
Dept. of Physiology, Anat. and Genet., Univ. of Oxford, Oxford, United Kingdom

Abstract: Parkinson’s disease (PD) is a neurodegenerative disorder that affects approximately 1% of those aged over 65. Accumulating evidence links oligomeric species of the protein alpha-synuclein to neuronal death associated with PD. However, the direct detection of alpha-synuclein oligomers in post-mortem brain has been challenging and this has limited our understanding of their structure, distribution and effects in the PD brain. Therefore, we have developed a novel method for the detection of alpha-synuclein oligomers, the alpha-synuclein proximity ligation assay (AS-PLA), which we demonstrated is specific for alpha-synuclein oligomers *in vitro*. Subsequently, in a blinded study with brain tissue from PD patients (n=8) and age- and sex-matched controls (n=8), we have shown that AS-PLA reveals widespread oligomeric alpha-synuclein pathology. AS-PLA preferentially detected early-stage, loosely compacted PD lesions such as pale bodies in patient tissue, whereas Lewy bodies, considered heavily compacted late lesions were only very exceptionally stained. AS-PLA detected four times as many pale bodies

as alpha-synuclein immunohistochemistry (AS-IHC) suggesting that AS-PLA is more sensitive to early PD lesions. Furthermore, we observed previously unidentified pathology in the form of distinctive AS-PLA diffuse staining. This type of staining was often localised to neuroanatomical regions mildly affected in PD, such as the cingulate cortex and the reticular formation of both the medulla and midbrain, in the absence of Lewy bodies. This contrasts with previously described methods for the detection of alpha-synuclein oligomers and conventional AS-IHC that strongly stain Lewy bodies, but reveal little other pathology. Diffuse AS-PLA staining was significantly more abundant in patients than controls in the medulla (3.7 fold increase) and cingulate cortex (1.6 fold increase). No difference was observed in the midbrain, possibly due to the extent of degeneration in this region in PD patients. The oligomeric species detected by AS-PLA displayed a unique, intermediate proteinase K (PK) resistance profile, suggesting they are early aggregates. Highly aggregated proteins display resistance to PK treatment, and the PK resistance of AS-PLA detected species fell between the PK sensitivity of normal synaptic alpha-synuclein and the PK resistance of Lewy bodies detected by AS-IHC. Therefore, we suggest that AS-PLA reveals widespread, previously unrecognised oligomeric pathology. The unique insight into early PD pathology provided by AS-PLA will be valuable in understanding the role of alpha-synuclein oligomers in the initial disease processes in PD.

Disclosures: **R. Roberts:** None. **R. Wade-Martins:** None. **J. Alegre-Abarrategui:** None.

Nanosymposium

484. Alpha-Synuclein and LRRK2 Mechanisms in Parkinson's Disease

Location: 146C

Time: Tuesday, November 18, 2014, 8:00 AM - 10:45 AM

Presentation Number: 484.07

Topic: C.03. Parkinson’s Disease

Support: NIH R01NS064934

U18NS082132

R01NS065860

Michael J. Fox Foundation for Parkinson’s Disease Research

American Parkinson’s Disease Association

Title: Defining the most efficient α-synuclein conformer for seeding pathologic inclusions

Authors: *L. A. VOLPICELLI-DALEY, M. MOEHLE, K. B. FRASER, H. ABDELMOTILIB, J. P. BLACKBURN, D. G. STANDAERT, A. B. WEST;
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Abstract: Parkinson's disease is pathologically characterized by Lewy bodies and Lewy neurites comprised of α-synuclein. Formation of these inclusions can be recapitulated in primary cultures and in the rodent brain by exposing neurons to α-synuclein amyloid fibrils. These fibrils gain entry to neurons by endocytosis and act as a template to induce the conversion of normal, soluble synuclein into aggregates that recapitulate many features of Lewy Bodies and Lewy Neurites in that they are hyperphosphorylated, insoluble, ubiquitinated, and filamentous by electron microscopy. However, the conformer of α-synuclein that can enter the neuron and seed the conversion normal α-synuclein is unknown. Neither monomeric nor fully fibrillized α-synuclein is capable of inducing inclusion formation, but smaller fragments are required. We use multiple high-resolution techniques to characterize and resolve the size, morphology, and structure of the α-synuclein seeds capable of templating conversion of normal α-synuclein into pathologic inclusions. These data will potentially help with identification of therapeutics to prevent the spread of α-synuclein aggregates.

Disclosures: L.A. Volpicelli-Daley: None. **M. Moehle:** None. **K.B. Fraser:** None. **H. Abdelmotilib:** None. **J.P. Blackburn:** None. **D.G. Standaert:** None. **A.B. West:** None.

Nanosymposium

484. Alpha-Synuclein and LRRK2 Mechanisms in Parkinson's Disease

Location: 146C

Time: Tuesday, November 18, 2014, 8:00 AM - 10:45 AM

Presentation Number: 484.08

Topic: C.03. Parkinson's Disease

Support: CIHR MOP298668

Title: Alpha-synuclein serine 129 phosphorylation modulates self-assembly and cellular uptake

Authors: *A. TANDON[1], F. SAMUEL[1], W. FLAVIN[2], C. PACELLI[3], L.-E. TRUDEAU[3], E. M. CAMPBELL[2], P. E. FRASER[1];
[1]Tanz Ctr. for Res. in Neurodegenerative Dis., Univ. of Toronto, Toronto, ON, Canada; [2]Loyola Univ., Chicago, IL; [3]Univ. of Montreal, Montreal, QC, Canada

Abstract: Aggregated α-synuclein (α-syn) is a primary component of Lewy bodies (LB) in Parkinson disease (PD). In healthy neurons, only a minute fraction of α-syn is phosphorylated at serine 129 (pS129), and is almost completely detergent-soluble, distributed between membrane and cytosolic fractions. However, 90% of α-syn isolated from LB is phosphorylated at S129 and is detergent-insoluble. In this study, we evaluated whether the nonphospho- and phosphoproteins display differential aggregation properties, membrane binding and cellular uptake. In vitro aggregation experiments revealed that pS129 promoted α-syn fibril formation regardless of the PD-linked mutation. Surprisingly, S129 phosphorylation of wild-type α-syn had no effect membrane binding, but rescued the weak membrane binding of A30P α-syn. However, characterization of α-syn uptake by cultured cell lines and by primary dopaminergic neurons suggested that internalization of sonicated preformed α-syn fibrils is enhanced by pS129, particularly of the A30P α-syn mutant. Lastly, using a lysosome disruption assay, we observed that permeability of lysosomal vesicle membranes was significantly increased by pS129 α-syn, particularly in conjunction with the A30P mutation. Overall, our results indicate that S129 phosphorylation has striking effects on the biophysical properties of α-syn, and potentiates it cellular accumulation and toxicity by promoting permeability of lysosomal vesicle membranes. The internalization of extracellular α-syn fibrils and access to endogenous cytosolic α-syn has implications for the cellular mechanisms the underlie the proposed prion-like neuron to neuron propagation of pathological α-syn in PD and other synucleinopathies.

Disclosures: A. Tandon: None. **F. Samuel:** None. **P.E. Fraser:** None. **W. Flavin:** None. **E.M. Campbell:** None. **C. Pacelli:** None. **L. Trudeau:** None.

Nanosymposium

484. Alpha-Synuclein and LRRK2 Mechanisms in Parkinson's Disease

Location: 146C

Time: Tuesday, November 18, 2014, 8:00 AM - 10:45 AM

Presentation Number: 484.09

Topic: C.03. Parkinson’s Disease

Title: LRRK2 kinase inhibition reduces endogenous LRRK2 protein levels *in vivo*

Authors: *E. LOBBESTAEL, S. DEMAN, T. DE WIT, J.-M. TAYMANS, V. BAEKELANDT;
KU Leuven, Leuven, Belgium

Abstract: LRRK2 (Leucine-rich repeat kinase 2) kinase inhibitors are proposed as potential PD drugs as they are reported to counteract toxic effects of pathogenic LRRK2 mutants. Still many

ambiguities exist concerning their therapeutic effects. For example, pharmacological LRRK2 kinase inhibition reduces cellular LRRK2 phosphorylation to the same extent as several pathogenic LRRK2 mutations. In addition, it has recently been shown that the beneficial effect of LRRK2 kinase inhibition on LRRK2-induced toxicity can be attributed to reduced LRRK2 protein levels rather than inhibition of kinase activity (Skibinski et al. 2014 J Neurosci 34(2):418-433). To further define the molecular consequences of LRRK2 kinase inhibitor treatment, we have explored the long-term effects of pharmacological LRRK2 kinase inhibition. Treatment of stable LRRK2 overexpressing SH-SY5Y cells with different LRRK2 kinase inhibitors induced LRRK2 dephosphorylation via recruitment of protein phosphatase 1 (Lobbestael et al. 2013 BJ 456:119-128) prior to reduction of its protein levels. We confirmed these data for endogenous LRRK2 from rodent brain, where we observed LRRK2 dephosphorylation and reduced LRRK2 protein levels after systemic administration of LRRK2 kinase inhibitor. Further investigation of the effect of LRRK2 kinase inhibition on LRRK2 mutations in cell culture revealed that also the pathogenic mutant G2019S displayed dephosphorylation prior to reduction of protein levels. Treatment of an inhibitor-resistant LRRK2 variant did not affect LRRK2 phosphorylation and stability, indicating that our findings result from LRRK2-specific inhibitor effects. Altogether, our findings shed light on the 'long-term' effects of LRRK2 kinase inhibition and have important implications regarding the use of LRRK2 kinase inhibitors as therapeutic agent. Currently, we are investigating the mechanisms of the inhibitor-induced reduction of LRRK2 levels. Further insight in the relation between LRRK2 kinase activity and LRRK2 stability, may point to new therapeutic targets for PD.

Disclosures: E. Lobbestael: None. **S. Deman:** None. **T. De Wit:** None. **J. Taymans:** None. **V. Baekelandt:** None.

Nanosymposium

484. Alpha-Synuclein and LRRK2 Mechanisms in Parkinson's Disease

Location: 146C

Time: Tuesday, November 18, 2014, 8:00 AM - 10:45 AM

Presentation Number: 484.10

Topic: C.03. Parkinson's Disease

Support: Wellcome Trust Grant WT095010MA

Vera Down British Medical Association Research grant (to K. H.)

Title: The importance of LRRK2-RTN interaction in neurodegeneration

Authors: *J. J. NIXON-ABELL[1], D. C. BERWICK[1], V. A. SPAIN[1], C. BLACKSTONE[2], K. HARVEY[1];
[1]Pharmacol., UCL, London, United Kingdom; [2]Natl. Inst. of Neurolog. Disorders and Stroke, NIH, Bethesda, MD

Abstract: Endoplasmic reticulum (ER) function is key to protein and lipid biosynthesis, vesicular transport and calcium homeostasis. Interference with regular ER function (ER stress) characterises neurodegeneration in both hereditary spastic paraplegias (HSPs) and Parkinson's disease (PD). Leucine-rich repeat kinase 2 (LRRK2) and the ER-shaping reticulon proteins (RTNs) have been implicated in PD and HSPs respectively, with mutations in LRRK2 representing the largest genetic contributor to familial PD, whilst mutations in the reticulon gene RTN2 cause a severe form of HSP. Furthermore, an initial Y2H screen demonstrated interaction between the LRRK2 Roc-COR GTPase domain and reticulon isoforms RTN1C and RTN3B. Immunocytochemistry, protein co-immunoprecipitation and yeast two-hybrid assays were used to further characterise LRRK2-RTN interaction. We have demonstrated association between both proteins in yeast and mammalian cells, with the N-terminal region of the LRRK2 Roc domain responsible for interaction with RTNs. Crucially, familial PD mutations and the phosphorylation state of the LRRK2 Roc-COR domain were shown to modulate LRRK2-RTN interaction, with pathogenic R1441G/H and Y1699C mutations decreasing, and N1437H increasing interaction strength. Although the precise mechanisms are as yet unclear, our data suggests modulation of the LRRK2-RTN interaction as a potential novel pathomechanism of PD.

Disclosures: J.J. Nixon-Abell: None. **D.C. Berwick:** None. **V.A. Spain:** None. **C. Blackstone:** None. **K. Harvey:** None.

Nanosymposium

484. Alpha-Synuclein and LRRK2 Mechanisms in Parkinson's Disease

Location: 146C

Time: Tuesday, November 18, 2014, 8:00 AM - 10:45 AM

Presentation Number: 484.11

Topic: C.03. Parkinson's Disease

Support: Michael J Fox Foundation

EMBO fellowship (MBO ASTF 526-2012/Award)

Telethon (Grant no. GGP12237)

Title: A functional interplay between Leucine rich repeat kinase 2 and p21 activated kinase 6 in neuronal systems

Authors: *L. CIVIERO[1], M. CIRNARU[2], A. BEILINA[3], U. RODELLA[1,4], I. RUSSO[1], E. BELLUZZI[1], E. LOBBESTAEL[4], L. REYNIERS[4], P. LEWIS[5,6], C. VAN DEN HAUTE[4,7], R. BANDOPADHYAY[8], V. BAEKELANDT[4], L. BUBACCO[1], G. PICCOLI[2], M. COOKSON[3], J. TAYMANS[4], E. GREGGIO[1];
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Abstract: Leucine rich repeat kinase 2 (LRRK2) is a large multidomain protein with GTPase and serine-threonine kinase domains. Mutations in LRRK2 are the most common genetic cause of Parkinson disease (PD) and synaptic dysfunction of the nigrostriatal pathway is a major disease hallmark. Although several efforts have been spent to understand LRRK2 pathological outcomes, little is known about its physiological role within nervous cells. Emerging evidence suggests that LRRK2 is associated with cytoskeletal components and influences vesicular trafficking, neurite outgrowth and synaptogenesis, pathways that are relevant for neuronal physiology. The p21 activated kinases (PAK) are a class of enzymes that act downstream of small GTPases in different morphogenetic processes through remodeling of the actin cytoskeleton and, in the context of the nervous system, are key factors for synapse and branching formation. Here, we have collected data showing that i) LRRK2 forms a functional complex with p21-activated kinase 6 (PAK6) in vitro and in vivo, ii) LRRK2:PAK6 complex impacts actin cytoskeleton dynamics and neurite growth, iii) at a molecular level, the kinase activity of PAK6 negatively regulates LRRK2 phosphorylation state and iv) pharmacological inhibition of PAKs significantly reduces mutant LRRK2-associated protein accumulations in cells. Further observations indicate that PAK6 is hyper-phosphorylated in brains of LRRK2-linked and idiopathic PD patients. Therefore, our findings describe a new LRRK2-PAK6 pathway in neurons and suggest novel avenues for therapeutics.

Disclosures: L. Civiero: None. **M. Cirnaru:** None. **A. Beilina:** None. **U. Rodella:** None. **I. Russo:** None. **E. Belluzzi:** None. **E. Lobbestael:** None. **L. Reyniers:** None. **P. Lewis:** None. **C. Van den Haute:** None. **R. Bandopadhyay:** None. **V. Baekelandt:** None. **L. Bubacco:** None. **G. Piccoli:** None. **M. Cookson:** None. **J. Taymans:** None. **E. Greggio:** None.

Nanosymposium

485. Epilepsy: Circuits, Mechanisms, and Potential Therapies

Location: 144A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 485.01

Topic: C.07. Epilepsy

Support: VA BLR&D BX002305

Title: Evidence for use-dependent and presynaptic actions of 2DG on abnormal synaptic network activity in the CA3 region of the hippocampus

Authors: Y.-Z. PAN[1], T. SUTULA[1], *P. A. RUTECKI[1,2];
[1]Neurol., Univ. of Wisconsin, Madison, WI; [2]William Middleton Mem. VA Hosp., Madison, WI

Abstract: 2-deoxy-D-glucose (2DG), a glucose analog that inhibits glycolysis, has acute and chronic antiepileptic effects. Acutely, 2DG (10 mM) decreases epileptiform activity evoked by a variety of mechanisms in the hippocampal slice (Stafstrom, et al, Ann Neurol, 2009). We studied the effects of 2DG on synaptic activity in the CA3 region of the rat hippocampal slice in normal and elevated potassium conditions using whole-cell voltage-clamp recordings of visually identified CA3 neurons. In control 3.5 mM $[K^+]_o$ artificial cerebrospinal fluid (ACSF), 2DG had no effects on the amplitude or frequency of spontaneously occurring EPSCs. In the presence of 7.5 mM $[K^+]_o$, EPSCs occurred more frequently and CA3 neurons demonstrated synchronous bursts of EPSCs that occurred in a rhythmic fashion. 2DG decreased the area of the EPSC inward current (the inward charge) measured at a holding potential of -70 mV by 77%. The net outward charge measured at 0 mV that represents charge associated with IPSCs was only reduced by 54% following 2DG application. In a separate series of experiments, miniature EPSCs (mEPSCs) were studied in 7.5 mM $[K^+]_o$ and tetrodotoxin (TTX 1 μM). 2DG was applied either before or after TTX. When applied during 7.5 mM $[K^+]_o$ induced increased synaptic activity and before TTX , the frequency, but not amplitude, of mEPSCs was significantly reduced compared to neurons treated with 2DG after TTX application. When 2DG was applied after TTX in 7.5 mM $[K^+]_o$ ACSF, there was no effect on the frequency or amplitude of mEPSCs. 2DG did not have an effect on mIPSC amplitude or frequency when applied before or after TTX application. These results demonstrate an antiepileptic effect of 2DG on abnormal epileptiform synaptic network activity produced by 7.5 mM $[K^+]_o$, a concentration that occurs during interictal and ictal synchronization, but not on normal activity in 3.5 mM $[K^+]_o$. The antiepileptic effect was associated with more prominent reduction of synchronous excitatory synaptic activity compared to the effect on inhibitory network synchronization. Furthermore, the effects of 2DG on EPSCs in 7.5 mM $[K^+]_o$ but not in 3.5mM $[K^+]_o$ are consistent with use-dependent effects, and the reduction of mEPSC frequency suggest a presynaptic action on excitatory transmitter release. The suppression of excitability on abnormal, but not normal,

network synaptic activity suggests that 2DG may be associated with a favorable side effect profile compared to conventional antiepileptic treatments.

Disclosures: Y. Pan: None. **T. Sutula:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); TS has equity in Neurogenomex. **P.A. Rutecki:** None.

Nanosymposium

485. Epilepsy: Circuits, Mechanisms, and Potential Therapies

Location: 144A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 485.02

Topic: C.07. Epilepsy

Support: NIH NINDSNS072179

EFA

NE LB692

Title: Consequences of seizure propagation to the sleep regulating lateral hypothalamus

Authors: *K. A. SIMEONE;
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Abstract: Sleep disorders are a prevalent comorbid condition in epilepsy. Symptoms, including inappropriate sleep-wake transitions and reduced sleep efficiency, are shared by sleep disorders in the non-epileptic population and associated with a dysregulation of orexin neurotransmission from the lateral hypothalamus. Orexin activates the ascending reticular activating system to stimulate wakefulness and arousal. We hypothesized that the orexin system is hyperactive in Kcna1-null mice, a clinically relevant model of temporal lobe epilepsy with comorbid sleep disorders. We investigated the pathology of the orexinergic system in the lateral hypothalamus (LH) of Kcna1-null mice, which have approximately seven seizures a day. EEG analyses indicated that the more severe seizures propagate to the LH. Immunohistochemical assessment indicates that injury is apparent in the LH, as determined by increased blood-brain-barrier permeability, astrogliosis and impaired mitochondrial bioenergetics. Astrogliosis and impaired mitochondria are both upstream of reduced adenosine tone. Adenosine is responsible for inhibiting orexin and promoting sleep. Multielectrode extracellular recordings of the spontaneously firing orexin neurons indicate a reduced response to adenosine receptor agonists

and antagonists, which suggests reduced adenosine inhibition of orexin in Kcna1-null LH. Collectively, these data suggest Kcna1-null mice have a hyperactive orexin system that may be responsible for their reduced NREM and sleep efficiency.

Disclosures: K.A. Simeone: None.

Nanosymposium

485. Epilepsy: Circuits, Mechanisms, and Potential Therapies

Location: 144A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 485.03

Topic: C.07. Epilepsy

Support: NIH NINDS

Title: Stereological EM evidence that rapamycin nonspecifically blocks excitatory synaptic reorganization in a mouse model of temporal lobe epilepsy

Authors: *P. BUCKMASTER, R. YAMAWAKI, K. THIND;
Dept Comparative Med., Stanford Univ., Stanford, CA

Abstract: Rapamycin blocks granule cell axon (mossy fiber) sprouting after epileptogenic injuries, including pilocarpine-induced status epilepticus (SE). Accordingly, rapamycin reduces aberrant Timm staining in the inner molecular layer of the dentate gyrus. However, only mossy fibers are darkly labeled by Timm staining. Therefore it remains unclear whether axons from other neuronal types sprout into the inner molecular layer and synapse with granule cell dendrites despite rapamycin treatment. If so, aberrant positive-feedback networks might develop involving granule cells and surviving mossy cells and CA3 pyramidal cells. To test this possibility we used stereological electron microscopy to estimate numbers of excitatory synapses in the inner molecular layer per hippocampus in pilocarpine-treated control mice and in mice 5 d after SE to measure the maximal extent of acute loss. Other mice were evaluated after SE and daily treatment beginning 24 h later with 3 mg/kg rapamycin or vehicle for 2 months (3 mice/group). Serial electron micrographs (50/site) were evaluated at 6 random sites in the inner molecular layer per hippocampus. Numbers of asymmetric synapses were measured and used to estimate the total number per hippocampus, which was 1.09 ± 0.03 billion (mean ± sem) in controls. 5 d after SE average synapse number was reduced to 59% of controls (p=0.001). 2 mo after SE synapse number recovered to 101% of controls in vehicle-treated mice but was only 70% of controls in rapamycin-treated mice (p=0.006). Stereological methods were used to estimate

numbers of granule cells in Nissl-stained sections from the same mice so that numbers of excitatory synapses in the inner molecular layer per granule cell could be calculated. Control mice had 484,000 ± 36,000 granule cells/hippocampus. Average granule cell numbers were 95% of controls in mice 5 d after SE, 108% in vehicle-treated mice 2 mo after SE, and 109% in rapamycin-treated mice. Control mice had 2280 ± 230 excitatory synapses in the inner molecular layer per granule cell, which was reduced to 63% of controls 5 d after SE ($p<0.05$), recovered to 93% at 2 mo (vehicle-treated), and was only 63% in rapamycin-treated mice ($p<0.05$). These findings reveal that rapamycin blocked development of excitatory synapses after pilocarpine-induced SE. Mossy fibers and other excitatory axons were prevented from synapsing with proximal dendrites of granule cells. Together with previous findings that rapamycin treatment does not affect development of spontaneous seizures, these findings suggest excitatory synaptic reorganization in the dentate gyrus is not necessary for temporal lobe epileptogenesis.

Disclosures: P. Buckmaster: None. **R. Yamawaki:** None. **K. Thind:** None.

Nanosymposium

485. Epilepsy: Circuits, Mechanisms, and Potential Therapies

Location: 144A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 485.04

Topic: C.07. Epilepsy

Support: NIH Grant NS040272

NIH Grant NS085046

Title: Disrupted synaptic E/I balance in a mouse model of human lissencephaly

Authors: *R. F. HUNT, M. T. DINDAY, K. M. GIRSKIS, S. C. BARABAN;
Neurolog. Surgery, Univ. of California, San Francisco, San Francisco, CA

Abstract: Hemizygous mutations in the PAFAH1B1 gene (often referred to as Lis1) can cause lissencephaly associated with Miller-Dieker syndrome, a devastating malformation of cortical development. Children with this disease suffer from severe intellectual disability, intractable epilepsy, and early death. While disruptions in neuronal migration are considered a primary cause of lissencephaly, how PAFAH1B1 mutations alter the function of individual subpopulations of neurons and ultimately brain circuits is largely unknown. In a recent study, we showed PAFAH1B1 mutation was directly linked to enhanced glutamatergic synaptic drive onto

dentate gyrus granule cells, independent from defects in neuronal migration (Hunt et al., J Neuroscience, 2012). To address how GABAergic circuits are altered in lissencephaly, we used mice with germline mutation of PAFAH1B1 or conditional deletion from inhibitory interneurons. Migration of young GABA neurons into the embryonic neocortex and hippocampus was slowed in mutant mice. At P30, mutant mice had significantly fewer GABA neurons in the dentate gyrus compared to wildtype littermates. Patch-clamp recordings obtained from granule cells revealed an increase in excitatory input and decreases in synaptic inhibition when measured in the same cell. In addition, using a battery of behavioral tests and long-term video-EEG monitoring, we are investigating the effect of conditional PAFAH1B1 mutation in different cell types and at different stages of development. Our results suggest brain region-specific impairments in GABA neuron migration and function may contribute to behavioral disturbances associated with PAFAH1B1 mutations.

Disclosures: R.F. Hunt: None. **M.T. Dinday:** None. **K.M. Girskis:** None. **S.C. Baraban:** None.

Nanosymposium

485. Epilepsy: Circuits, Mechanisms, and Potential Therapies

Location: 144A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 485.05

Topic: C.07. Epilepsy

Support: Wellcome Trust

European Research Council

Title: DREADD-mediated attenuation of focal neocortical seizures

Authors: D. KAETZEL[1], E. NICHOLSON[1], S. SCHORGE[1], M. C. WALKER[1], *D. M. KULLMANN[2];
[1]UCL, London, United Kingdom; [2]Inst. Neurology, UCL, London WC1N 3BG, United Kingdom

Abstract: Focal epilepsy is commonly pharmacoresistant, and resective surgery is often contraindicated by proximity to eloquent cortex. Many patients have no effective treatment options. Gene therapy allows cell-type specific inhibition of neuronal excitability, but on-demand seizure suppression has only been achieved with optogenetics, which requires invasive light delivery. We tested a combined chemical-genetic approach using a Designer Receptor

Exclusively Activated by a Designer Drug (DREADD) to achieve localized suppression of neuronal excitability in a seizure focus. We used viral expression of the modified Gi-coupled muscarinic receptor hM4Di, which is activated by the normally inactive and orally bioavailable agonist clozapine-N-oxide (CNO). hM4Di was expressed either in the motor cortex or in the hippocampus using an adeno-associated viral vector under the Camk2a promoter. EEG was recorded using a wireless subcutaneous transmitter (Open Source Instruments Inc). Hippocampal hM4Di attenuated field EPSPs recorded in acute hippocampal slices in the presence of CNO, showing that it depresses neurotransmitter release in addition to its known hyperpolarizing effect mediated by GIRKs. hM4Di in the motor cortex had no detectable effect on a sensorimotor coordination task in the presence or absence of CNO administered by intraperitoneal injection. hM4Di-CNO suppressed focal seizures evoked by either pilocarpine or picrotoxin injected into the motor cortex. hM4Di-CNO also had a robust anti-seizure effect in a chronic model of focal neocortical epilepsy induced by tetanus toxin injection into the motor cortex. Chemical-genetic seizure attenuation holds promise as a novel approach to treat intractable focal epilepsy while minimizing disruption of normal circuit function in untransduced brain regions or in the absence of the specific ligand. Although this approach cannot match the temporal specificity of optogenetics it avoids the need to express a non-mammalian protein and deliver light to the seizure focus.

Disclosures: D. Kaetzel: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); DK, SS, MCW and DMK have applied for a patent relating to the use of DREADDs in the treatment of epilepsy. **D.M. Kullmann:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); DK, SS, MCW and DMK have applied for a patent relating to the use of DREADDs in the treatment of epilepsy. **E. Nicholson:** None. **S. Schorge:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); DK, SS, MCW and DMK have applied for a patent relating to the use of DREADDs in the treatment of epilepsy. **M.C. Walker:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); DK, SS, MCW and DMK have applied for a patent relating to the use of DREADDs in the treatment of epilepsy.

Nanosymposium

485. Epilepsy: Circuits, Mechanisms, and Potential Therapies

Location: 144A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 485.06

Topic: C.07. Epilepsy

Support: National Institutes of Health

PSU Stem Cell Endowment Fund

Title: Directly convert NG2 cells into functional GABAergic neurons

Authors: *Z. GUO, Y. CHEN, Z. PEI, G. CHEN;
Dept. of Biol., Pennsylvania State Univ., University Park, PA

Abstract: Our previous studies have demonstrated that overexpression of a single neural transcription factor NeuroD1 can efficiently transdifferentiate reactive glial cells into fully functional neurons, which can successfully integrate into the local neural circuitry in adult mouse cortex (Guo et al., 2014, Cell Stem Cell). Following expression of NeuroD1, astrocytes were reprogrammed into glutamatergic neurons, while NG2 cells were reprogrammed into glutamatergic and GABAergic neurons. The direct conversion of endogenous astrocytes into functional neurons makes it possible to develop new therapy for brain repair. Epilepsy is a severe neurological disorder majorly caused by GABAergic dysfuction. Even though NeuroD1 can reprogram NG2 cells into GABAergic neurons, the efficiency is relatively low. Therefore, we screened a pool of transcriptional factors and found that NeuroD1 together with Dlx2 can efficiently convert mouse NG2 cells into mature GABAergic neurons. Importantly, some of NG2-converted GABAergic neurons can fire fast-spiking action potentials. Furthermore, we demonstrated that human OPCs can also be efficiently converted into functional GABAergic neurons by overexpressing NeuroD1 plus Dlx2. Our studies therefore suggest that direct reprogramming of NG2 cells into functional GABAergic neurons might provide an alternative approach for the treatment of epileptic brain.

Disclosures: **Z. Guo:** None. **Y. Chen:** None. **Z. Pei:** None. **G. Chen:** None.

Nanosymposium

485. Epilepsy: Circuits, Mechanisms, and Potential Therapies

Location: 144A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 485.07

Topic: C.07. Epilepsy

Support: MRC

Title: GABA(A) receptor trafficking in hippocampal neurons during status epilepticus

Authors: *R. ECKEL[1,2], J. KITTLER[1], M. WALKER[2];
[1]Neuroscience, Physiol. and Pharmacol., Univ. Col. London, London, United Kingdom; [2]Dept. of Clin. and Exptl. Epilepsy, Inst. of Neurol., London, United Kingdom

Abstract: Gamma - aminobutyric acid type A receptors ($GABA_A$Rs) are the most abundant inhibitory neurotransmitter receptors in the mammalian brain. They are assembled from various subunits forming heteropentamers that contain a binding pocket for benzodiazepines between α and γ subunits. During the prolonged, self – sustained seizures of Status Epilepticus (SE), pharmacoresistance to benzodiazepines develops progressively. $GABA_A$Rs have been shown to undergo subunit – specific down – modulation in models of SE and could therefore play an important role in benzodiazepine pharmacoresistance. Despite recent studies investigating the modified trafficking of $GABA_A$Rs during SE, the molecular mechanisms remain poorly understood. We performed a live - cell imaging study to investigate the trafficking properties and mechanisms underlying inhibitory plasticity in an *in vitro* model of SE. Epileptiform activity of hippocampal neurons in culture was driven by perfusion with Mg^{2+} - lacking aCSF (low Mg^{2+}). By live - cell imaging of super ecliptic pHluorin (SEP) – tagged α_2 containing $GABA_A$Rs we show they undergo surface down – modulation dependent on N-methyl-D-aspartate receptor (NMDAR) activity. Interestingly, we report that this down modulation, which primarily occurs in the soma, correlates well with intracellular Ca^{2+} changes. Moreover, it is inhibited by blocking the phosphatase calcineurin with an autoinhibitory peptide. Thus, NMDAR signalling via calcineurin could have important implications for benzodiazepine pharmacoresistance. This work provides new insights into our understanding of the molecular regulation of $GABA_A$R trafficking during SE.

Disclosures: **R. Eckel:** None. **J. Kittler:** None. **M. Walker:** None.

Nanosymposium

485. Epilepsy: Circuits, Mechanisms, and Potential Therapies

Location: 144A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 485.08

Topic: C.07. Epilepsy

Support: ANR-10-LABX-0087 IEC

ANR-10-IDEX-0001-02 PSL

Title: Effects of a reduced efficacy of the KCC2 co-transporter and its relevance for epilepsy

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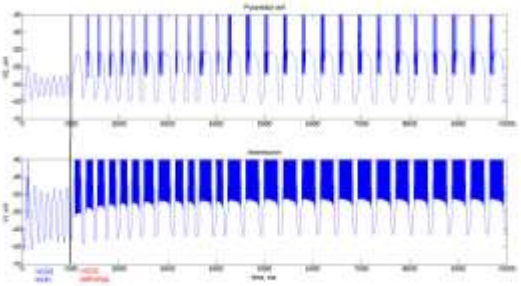
Abstract: Epilepsy is one of the most common neurological disorders. Despite much research seizures in about 40% of patients are pharmaco-resistant. Surgical removed focal tissue from these patients may be used for studies on the pathological mechanisms underlying seizures. Thus in the human epileptogenic subiculum the KCC2 cotransporter is absent or non-functional in about 20 % of pyramidal cells. This molecule normally assures the homeostatic maintenance of intra-neuronal chloride levels. Chloride concentration changes in pyramidal neurons caused by intensive GABAergic input during seizures could reverse the effects of GABA currents from inhibitory to excitatory. Such changes may shift pyramidal cells into the bursting regime associated with the clonic phase of seizures. Using a biophysical model of a single cell and a neural population model representing a simple network we show that that decreasing the activity of KCC2 pump leads to repetitive seizure-like firing in the epileptogenic focus. This model provides the insights into how the functional pathology of pyramidal cells associated with the absence of the KCC2 co-transporter leads to seizures in the epileptogenic human Subiculum.



Disclosures: **A. Buchin:** None. **B. Gutkin:** None. **G. Huberfeld:** None. **R. Miles:** None. **A. Chizhov:** None.

Nanosymposium

485. Epilepsy: Circuits, Mechanisms, and Potential Therapies

Location: 144A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 485.09

Topic: C.07. Epilepsy

Support: NIH Grant R01 NS25704

Title: Synergistic effects of impaired electrical excitability of parvalbumin- and somatostatin-expressing interneurons by selective deletion of Nav1.1 channels in a mouse model of Dravet syndrome

Authors: ***M. RUBINSTEIN**, C. TAI, R. E. WESTENBROEK, T. SCHEUER, W. A. CATTERALL;
Pharmacol., Univ. of Washingtin, Seattle, WA

Abstract: Dominant loss-of-function mutations in voltage-gated sodium channel Nav1.1 cause the intractable childhood-onset epilepsy Dravet Syndrome (DS). Previous studies using a mouse model of DS demonstrated impaired excitability of GABAergic interneurons in the cerebral cortex and hippocampus. Here we investigate the cellular and physiological consequences of selective deletion of Nav1.1 in parvalbumin-expressing (PVds) or somatostatin-expressing (SSTds) interneurons. In slices of cerebral cortex, haploinsufficiency of Nav1.1 in PVds or SSTds interneurons resulted in increased threshold and rheobase for action potential (AP) generation, and reduced number and frequency of AP during trains. In hippocampal slices, recordings from CA1 stratum oriens OL-M cells, which express both PV and SST, also demonstrated increased threshold and rheobase, and increased threshold for synaptically evoked AP as well as shortening of the excitatory postsynaptic potential. Selective haploinsufficiency of Nav1.1 in either PV or SST interneurons was sufficient to confer sensitivity to thermally induced seizures at postnatal day (P) 35. Mice with combined deletion of Nav1.1 in both PV and SST interneurons (PVds+SSTds) demonstrated thermally induced seizures as early as P21. Moreover, at P35, seizure duration in PVds+SSTds mice was ~6-fold longer than in PVds mice and ~2-fold longer than in SSTds mice. Homozygous deletion of Nav1.1 in PV neurons alone resulted in seizure induction at lower temperatures and premature death. In contrast, neither heterozygous nor homozygote deletion of Nav1.1 in SST interneurons caused premature death. Together, our results reveal synergistic effects of Nav1.1 haploinsufficiency in PV- and SST-expressing interneurons on seizure susceptibility in DS mice. R.M. and T.C contributed equally to this work.

Disclosures: **M. Rubinstein:** None. **C. Tai:** None. **R.E. Westenbroek:** None. **T. Scheuer:** None. **W.A. Catterall:** None.

Nanosymposium

485. Epilepsy: Circuits, Mechanisms, and Potential Therapies

Location: 144A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 485.10

Topic: C.07. Epilepsy

Title: Epilepsy associated mutant voltage-gated sodium channels alter resurgent current generation that could be preferentially targeted with cannabidiol

Authors: *R. PATEL[1], C. BARBOSA[2], T. R. CUMMINS[1];
[1]Stark Neurosci. Res. Inst., [2]Dept. of Pharmacol. and Toxicology, IU Sch. of Med., Indianapolis, IN

Abstract: Mutations in brain isoforms of voltage-gated sodium channels (VGSCs) have been identified in patients with distinct epileptic phenotypes. Most of these mutations occur in SCN1A (Nav1.1) and are associated with severe myoclonic epilepsy in infancy also known as Dravet Syndrome. Recently, mutations in SCN8A (Nav1.6) have been identified in patients with severe epileptic encephalopathy. Clinically, these patients do not respond well to classical antiepileptics and many remain refractory to treatment. Exogenous as well as endogenous cannabinoids have been shown to target VGSCs and has recently received attention for its efficacy in the treatment of childhood epilepsies. In this study, we wanted to further explore how cannabinoids modulate sodium currents from wildtype and epilepsy-associated mutant VGSCs. We examined the biophysical consequences of epilepsy-associated missense mutations in both Nav1.1 (R1648H and N1788K) and Nav1.6 (N1768D and L1331V), some of which have not been previously characterized. We used site-directed mutagenesis to introduce the missense mutations into the cDNA constructs of wildtype channels. Hek293t cells were transfected with wildtype or mutant channels using a calcium-phosphate transfection method and whole-cell patch clamp recordings were obtained in the presence and absence of 200μM ß4 peptide in the internal solution to induce resurgent sodium currents. Resurgent sodium currents are an atypical subthreshold current that is thought to influence cellular excitability and has been implicated in many disorders of excitability. We found that both mutations in Nav1.1 decreased resurgent currents while both mutations in Nav1.6 increased resurgent currents. We then examined the effects of anandamide, N-arachidonoylglycine, and cannabidiol (CBD) on transient and resurgent currents from wildtype and mutant channels. Interestingly, we found that CBD can preferentially target resurgent sodium currents over peak transient currents generated by Nav1.6. Targeting resurgent sodium current is a novel therapeutic strategy for the treatment of childhood epilepsies and although further studies are needed, these results support the potential use of CBD in the treatment of childhood epilepsies.

Disclosures: R. Patel: None. **C. Barbosa:** None. **T.R. Cummins:** None.

Nanosymposium

485. Epilepsy: Circuits, Mechanisms, and Potential Therapies

Location: 144A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 485.11

Topic: C.07. Epilepsy

Support: NIH Grant K08NS069783

Title: *In vivo* modulation of synaptic noise: A novel model of ictogenesis

Authors: *W. C. STACEY[1], P. STARSKI[2], H. LUNA-MUNGUIA[1];
[1]Neurol., Univ. of Michigan, Ann Arbor, MI; [2]Mayo Clin., Rochester, MN

Abstract: Despite a decade of research, we still have very limited understanding of how seizures are formed. This limits our ability to identify ictal biomarkers and understand how seizures initiate and spread. We present here a novel model of in vivo ictogenesis. Our theoretical and in vitro work suggest that one potential trigger for endogenous seizures is an acute increase in afferent synaptic activity, or "synaptic noise." We recently demonstrated in the intact septo-hippocampal formation that synaptic noise is sufficient to induce hippocampal seizures. Here, we use this strategy in pilocarpine rats to control the timing of seizure onset. Focal microinjections of KCl or bicuculline into a remote brain nucleus triggered hippocampal seizures in 6 rats. Frontal and hippocampal depth electrodes were used on a continuous EEG monitoring system to identify synaptic activity. Seizure activity was quantitatively and qualitatively similar to the spontaneous seizures, and occurred within 1 minute of an injection. This model allows direct control of endogenous seizure onset and has great potential for future research into seizure mechanisms and biomarkers.

Disclosures: W.C. Stacey: None. **P. Starski:** None. **H. Luna-Munguia:** None.

Nanosymposium

485. Epilepsy: Circuits, Mechanisms, and Potential Therapies

Location: 144A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 485.12

Topic: C.07. Epilepsy

Support: NIH grant NS084142-01

Title: Rapid recovery of action potential firing after cessation of human focal seizures

Authors: *E. H. SMITH[1], R. D. CONNORS[2], L. M. BATEMAN[2], C. A. SCHEVON[2]; [1]Neurosurg., [2]Neurol., Columbia Univ., New York, NY

Abstract: Despite over 50 years of epilepsy research, little is known about how seizures stop or how brain activity recovers postictally. It was recently proposed that neuronal synchronization increases until seizure termination, at which point the neurons are thought to become exhausted. These exhausted neurons could be involved with commonly observed postictal maladies including clinical depression, psychosis, hemiparesis, or sudden unexpected death in epilepsy (SUDEP). SUDEP's incidence rate is 0.1% per year, yet its mechanisms are not understood. Postictal generalized electroencephalographic suppression (PGES) is often observed after generalized tonic-clonic seizures, which are most commonly associated with SUDEP. The presence of PGES has been taken as evidence of a widespread "cerebral electrical shutdown," which has been implicated as a mechanism for SUDEP. This study was designed to test two related hypotheses: First, that neurons become exhausted as seizures approach termination, and second, that the cerebral cortex is inactive after seizures long enough to explain durations of postictal clinical symptoms, which can last for hours. To test these hypotheses, we examined electrophysiological recordings from penetrating microelectrode arrays implanted into human patients undergoing seizure monitoring for surgical treatment of pharmacoresistant epilepsy. We compared preictal and postictal measures of multiunit action potential firing for 60-second epochs before and after 11 seizures recorded from the seizure focus in 5 patients, and 11 seizures recorded from the ictal penumbra in 3 patients. This study shows that during a seizure, multiunit firing rate increases per burst as a seizure progresses towards termination, suggesting that neurons do not become exhausted toward the end of seizures. We found no significant difference between preictal and postictal firing rate or firing rate variance in either the seizure focus or the ictal penumbra. We found no significant difference between mutual information for preictal and postictal action potential times in the ictal penumbra. However, mutual information for action potential times among combinations of microelectrode array channels significantly increased postictally ¬in the seizure focus. This altered firing pattern may explain the loss of neurological function postically, as being mediated by disrupted neuronal communication. This study provides evidence that neurons do not get exhausted at the end of a seizure, and that neurons return to preictal firing rates rapidly after seizures stop. Further, this study provides a poignant counterexample for the theory of cerebral electrical shutdown.

Disclosures: E.H. Smith: None. **R.D. Connors:** None. **L.M. Bateman:** None. **C.A. Schevon:** None.

Nanosymposium

485. Epilepsy: Circuits, Mechanisms, and Potential Therapies

Location: 144A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 485.13

Topic: C.07. Epilepsy

Support: Rudi Schulte Research Institute, NSF (BMES-ERC)

DARPA (REMIND Project)

Title: Unstable periodic orbits of interictal bursts in hippocampal slices from patients with epilepsy

Authors: *P.-N. YU, M.-C. HSIAO, D. SONG, C. Y. LIU, C. N. HECK, D. MILLETT, T. W. BERGER;
USC, Los Angeles, CA

Abstract: Epilepsy is one of the most common neurological disorders; about 3% of all people living to the age of 60 will be diagnosed with epilepsy. Deep brain stimulation and responsive neurostimulation are rapidly growing treatment to abort epilepsy by interfering the dynamics of seizure with electrical stimulation. Although the preliminary results of them are promising, the underlying mechanism of dynamics is still unclear. Unstable periodic orbit (UPO), which is the skeleton of chaotic dynamics, provides us an insight into the dynamics of epilepsy. In this study, UPOs of interictal activity in hippocampal slices from patients with epilepsy have been detected and validated. Extracellular field potential recording results show that interictal activity can be induced in granule cell layer of dentate gyrus (DG) with high-potassium (8 mM), low-magnesium (0.25 mM) and 100uM 4-Aminopyridine (4-AP) artificial cerebrospinal fluid. Inter-pulse interval was used to reconstruct the dynamics of interictal activity. In addition, UPOs have been detected by periodic transform method. Surrogate analysis shows that UPOs are not due to noise with 95% confidence level. Short-term prediction of interictal interval around UPO will also be shown. This advance in understanding of epilepsy dynamics may facilitate the control of epilepsy.

Disclosures: **P. Yu:** None. **M. Hsiao:** None. **D. Song:** None. **C.Y. Liu:** None. **C.N. Heck:** None. **D. Millett:** None. **T.W. Berger:** None.

Nanosymposium

486. Traumatic Brain Injury: Therapeutic Strategies I

Location: 156

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 486.01

Topic: C.10. Trauma

Title: TIMP3 activates the Akt-mTORC1 pathway, imparts neuroprotection and attenuates neurocognitive dysfunction following Traumatic Brain Injury

Authors: *S. L. GIBB, S. PATI;
BSRI, San Francisco, CA

Abstract: Mesenchymal stem cells (MSCs) have been shown to have potent therapeutic effects in a number of neurological disorders involving neuronal loss and neuroinflammation including Traumatic Brain Injury (TBI). However, the molecular mechanism(s) underlying these protective effects are largely unknown. We hereby demonstrate that Tissue Inhibitor of Matrix metalloproteinase-3 (TIMP3), a soluble protein released by MSCs, is neuroprotective and enhances neurite outgrowth in vitro. Post-TBI intravenous TIMP3 treatment abrogates hippocampal loss of mature neurons, neural progenitors and dendritic projections in the dentate gyrus. Mechanistically we demonstrate that neuroprotection results from TIMP3-mediated activation of the Akt-mTORC1 pathway both in vitro and in vivo. Post-injury intravenous TIMP3 administration reduces anxiety-like behavior and improves hippocampal-dependent neurocognitition. Taken together our data strongly suggest that TIMP3 has neuroprotective effects that can mitigate the deleterious effects associated with TBI, an area with few if any therapeutic options.

Disclosures: S.L. Gibb: None. **S. Pati:** None.

Nanosymposium

486. Traumatic Brain Injury: Therapeutic Strategies I

Location: 156

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 486.02

Topic: C.10. Trauma

Support: Roskamp Foundation

DoD Grant W81XWH-13-1-0253

Title: Delayed treatment with Anatabine after repetitive mild TBI normalizes spatial memory impairment

Authors: *S. FERGUSON[1,2], B. MOUZON[1,2], L. ABDULLAH[1,2], G. CRYNEN[1], V. MATHURA[1], M. MULLAN[1,2], F. CRAWFORD[1,2];
[1]Roskamp Inst., Sarasota, FL; [2]James A Haley Veteran's Hosp., Tampa, FL

Abstract: Traumatic brain injury (TBI) is a serious illness which on average strikes one person every 15 seconds in the US. TBI carries long term consequences, even after mild TBI, which are the most common and comprise as many as 75% of all TBI cases. Despite the mild nature of the initial insult, secondary injury neuroinflammatory and neurodegenerative processes are initiated and continue for weeks and months afterward. Previously we reported on the potential of anatabine to affect neuroinflammation and improve memory at a long term timepoint when taken acutely after mild TBI. We have characterized anatabine's effects in a crossover study as a continuation of our previous work. We treated mice orally with anatabine in a closed head injury model of mild TBI. Anatabine was administered in their water starting 30 minutes after injury and continuing for 9 months. Untreated mice received regular water, and anesthesia controls were used for both the treated and untreated groups (r-sham). At a chronic timepoint 6 months after injury we saw a significant improvement of spatial memory of the anatabine treated r-mTBI mice compared to untreated r-mTBI mice, with anatabine treated r-mTBI mice performing as well as r-sham mice. At 9 months, 4 mice per group were euthanized for neuropathological analyses, revealing regionally-specific reductions in IBA1 and GFAP staining in the anatabine treated r-mTBI mice. We have continued to characterize the surviving mice using a crossover study design. Both r-mTBI and r-sham mice that were previously untreated were given anatabine starting at the 9 month timepoint. Mice that previously received anatabine began receiving regular water only. The mice were re-evaluated using the Barnes maze at 12 and 18 months post-injury. Although r-mTBI mice that began taking anatabine at 9 months post-TBI continued to perform worse than shams at the 12 month timepoint, by 18 months injured mice that had previously performed significantly worse than sham mice were now performing as well as shams. Pathological analysis of the brain tissue once again reveals regionally-specific differences that appear to be driven by anatabine treatment. Anatabine shows strong potential at improving memory following TBI, and may have a long therapeutic window, even to chronic timepoints; these data support further preclinical exploration of anatabine as a treatment for TBI. Dr. Michael Mullan is the CEO of Rock Creek Pharmaceuticals which sells anatabine as a nutraceutical supplement. The anatabine used in this study was provided by Rock Creek

Pharmaceuticals. None of the other authors receive remuneration from Rock Creek Pharmaceuticals. This study was funded by the Roskamp foundation.

Disclosures: **S. Ferguson:** None. **B. Mouzon:** None. **L. Abdullah:** None. **G. Crynen:** None. **V. Mathura:** None. **M. Mullan:** A. Employment/Salary (full or part-time):; Rock Creek Pharmaceuticals. **F. Crawford:** None.

Nanosymposium

486. Traumatic Brain Injury: Therapeutic Strategies I

Location: 156

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 486.03

Topic: C.10. Trauma

Support: Department of Veterans Affairs (Veterans Health Administration, Office of Research and Development, Rehabilitation Research and Development)

Bay Pines Foundation

Florida Department of Health James and Esther King Biomedical Research Program

Title: Withania somnifera extract protects model neurons from *in vitro* traumatic injury

Authors: ***J. N. CHANG**[1,2], H. HATIC[1], E. SHAW[3], V. RAVINDRANATH[3], B. A. CITRON[1,2];
[1]Res. & Develop., Bay Pines VA Healthcare Syst., Bay Pines, FL; [2]Mol. Med., Univ. of South Florida Morsani Col. of Med., Tampa, FL; [3]Ctr. for Neurosci., Indian Inst. of Sci., Bangalore, India

Abstract: Ayruvedic medicines have been used for millennia, but the efficacy and mechanisms are largely unknown. The root of the Withania somnifera plant has been used to treat several disorders including neurodegeneration and likely increases antioxidant capacities. We sought to determine whether this extract can protect cultured neurons from an in vitro injury that mimics a traumatic brain injury. Neuronal cultures were plated on silastic membranes and treated with extract from the W. somnifera root. A calibrated pressure pulse produced a transient biaxial stretch injury of the neurons to mimic the rotational forces that occur following traumatic brain injury. Neuronal health was evaluated at early, mid, and late stages of loss by staining with annexin, permeability to propidium iodide, and monitoring released lactate dehydrogenase activity. Neuronal processes were measured to examine neurite numbers and length. Quantitative

RT-PCR, Western blots, and protein arrays were performed to examine potential mechanistic changes in selected mRNAs and proteins. We found that pretreatment with W. somnifera root extract produced a significant decrease in annexin and PI staining following traumatic injury as well as a decrease in released lactate dehydrogenase activity. The treatment also benefited both the number and length of processes extending from neurons following the model traumatic injury. Significant differences were not found in the expression of the antioxidant transcription factor, Nrf2 or HSP70, but there was a reduction in apoptotic signaling factors produced by the treatment. Overall, W. somnifera extract was able to protect neurons from model traumatic brain injury in vitro. We will next further define the underlying molecular processes responsible for the neuroprotection observed in this system. Disclaimer: The contents do not represent the views of the Department of Veterans Affairs or the United States Government.

Disclosures: J.N. Chang: None. **H. Hatic:** None. **E. Shaw:** None. **V. Ravindranath:** None. **B.A. Citron:** None.

Nanosymposium

486. Traumatic Brain Injury: Therapeutic Strategies I

Location: 156

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 486.04

Topic: C.10. Trauma

Support: ITHS pilot funding

Title: Microtubule-stabilizing therapeutics improve long term outcome after traumatic brain injury

Authors: *D. J. CROSS, M. M. CLINE, G. G. GARWIN, L. HYSA, E. BRIM, S. MINOSHIMA;
Univ. of Washington, SEATTLE, WA

Abstract: Pharmacologic interventions for traumatic brain injury (TBI) may improve patient outcome, but no therapeutic has proven clinically effective to date. We previously applied microtubule-stabilizing drug, paclitaxel directly to TBI in rodents and showed improved short-term (8 days) functional outcome and reduced evidence of lesion on MRI. The goal of this study was to deliver paclitaxel (TAX) (limited BBB permeability) and an alternative drug Epothilone D (EpoD) (good BBB permeability), systemically and assess long term therapeutic effect. Methods: Mice (C57BL6 male, n=19, 10 wks) had craniotomy plus controlled cortical impact

(CCI) (Leica Biosystems), followed by 50 µl of vehicle (VEH) (n=5), 3 mg/kg of TAX (n=5), or 3 mg/kg of EpoD (n=4) via intraperitoneal injection. Sham surgery (craniotomy no CCI) was performed for control (SHAM) (n=5). At baseline and 32 days post surgery, subjects underwent neurological assessment (blinded) including grid test and CatWalk gait analysis (Noldus Information Technology) as well as MRI (14T MR Avance III, Bruker). Quantitative T2 maps were obtained and VOI analysis of lesion volume of edema was performed. Longitudinal and between group differences of neurological outcomes and VOIs were assessed ($p \leq 0.05$). Results: At 32d gait analysis revealed that VEH had contralateral impairment including print width (-6%) and print area (-15%) compared to SHAM and TAX had contralateral improvement over VEH (similar to SHAM values) including print area (22%, 0.67±0.16 vs 0.47±0.05cm2), print width (15%, 0.96±0.11 vs 0.83±0.06cm) and print length (23%, 1.15±0.14 vs 0.93±0.02cm)($p \leq 0.05$). EpoD indicated a more limited improvement over VEH in print length only (13%, 1.05±0.14 vs 0.93±0.02cm, $p \leq 0.05$). Grid test at 32d compared to baseline showed an increase (45%) in percent contralateral foot faults for VEH, however no significant increases were seen in SHAM, TAX or EpoD from baseline. Groupwise comparison at 32d indicated TAX and SHAM were decreased from VEH (61.6±14.9 and 58.1±11.6 respectively vs 77.7±8.5, $p \leq 0.05$), but EpoD group did not meet statistical threshold (68.0±15.1) compared to VEH. However, analysis of T2 maps indicated that EpoD reduced volume of edema by 52% (4.2±2.8 vs 8.74±3.2mm3, $p \leq 0.05$). No significant effect on edema was seen with TAX. These results indicate that administration microtubule-stabilizers following TBI improves functional outcome despite limited BBB permeability for TAX versus good permeability of EpoD. However, poor BBB penetration may limit direct effects on injury. Results from this research could impact the development of therapeutic options for the clinical management of traumatic brain injury.

Disclosures: **D.J. Cross:** None. **M.M. Cline:** None. **G.G. Garwin:** None. **L. Hysa:** None. **E. Brim:** None. **S. Minoshima:** None.

Nanosymposium

486. Traumatic Brain Injury: Therapeutic Strategies I

Location: 156

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 486.05

Topic: C.10. Trauma

Support: Department of Defense W81XWH-10-1-0494

Citizens United for Research in Epilepsy

Title: Ceftriaxone treatment after traumatic brain injury preserves GAD-1 expression in rat cortex after TBI

Authors: *M. Q. HAMEED, T. H. HSIEH, G. S. GOODRICH, J. L. MORALES-QUEZADA, P. A. ROSENBERG, A. ROTENBERG;
Neurol., Boston Children's Hosp., Boston, MA

Abstract: BACKGROUND: Traumatic brain injury (TBI) results in excessive regional glutamate release from dead and dying neurons, which contributes to excitotoxic cell injury and posttraumatic neurologic sequelae such as epilepsy. Glutamate transport is the only known mechanism for clearance of glutamate, largely via the membrane protein GLT1. We have shown that ceftriaxone (Cef) increases GLT1 expression after TBI, decreases regional gliosis, and suppresses posttraumatic seizures (Goodrich et. al., 2013). We also reported that there is a progressive loss of intracortical inhibition (ICI) as measured by paired pulse transcranial magnetic stimulation (ppTMS), along with increased oxidative stress and a gradual, selective loss of cortical parvalbumin interneurons in the weeks following LFPI (Lee et al., SFN 2013). Furthermore, we demonstrated that Cef treatment after injury preserves ICI and motor function in the acute period after motor cortex LFPI compared to saline controls (Hameed et al., SFN 2013). We now investigate whether this preservation of ICI corresponds to improved health and survival of cortical inhibitory interneurons. METHODS: Adult rats (n=20) received moderate lateral fluid percussion injury (LFPI; 2.3 ± 0.1 atm) via a craniotomy over the motor cortex, and were divided into 2 groups to receive Cef (200mg/kg/day IP, n=15) or saline (IP, n=15), daily, for one week after TBI. A sham group (n=5) received all surgical procedures except LFPI, and did not receive IP injections. Rats (N = 5) from each group were sacrificed 2 and 4 weeks after LFPI and RNA was extracted from fresh brain tissue. By real time PCR we measured GAD-1 expression in lesional and contralesional neocortex. RESULTS: At sampled timepoints, ipsilesional GAD-1 expression was significantly lower in saline-treated verum injury compared to sham controls ($p<0.05$). In contrast, rats treated with Cef after injury were no different than sham. Contralesional cortex was unaffected by injury. CONCLUSION: Taken together, the preservation of ICI and normalization of GAD-1 gene expression suggest preservation of GABAergic interneuron function as a mechanism for the anti-epileptogenic effect of Cef we reported previously (Goodrich et al., 2013). Although further work is ongoing, we cautiously conclude that the neuroprotective and anti-epileptogenic effect of ceftriaxone in the TBI setting can be tested in clinical trials, particularly given that Cef is a safe and already widely used drug.

Disclosures: M.Q. Hameed: None. **T.H. Hsieh:** None. **J.L. Morales-Quezada:** None. **P.A. Rosenberg:** None. **A. Rotenberg:** None. **G.S. Goodrich:** None.

Nanosymposium

486. Traumatic Brain Injury: Therapeutic Strategies I

Location: 156

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 486.06

Topic: C.10. Trauma

Support: SLL ALF

Swedish Defence Forces

Title: Neuroprotective effects of N-acetylcysteine amide on experimental focal penetrating brain injury in rats

Authors: *M. GÜNTHER[1], J. DAVIDSSON[2], S. PLANTMAN[1], M. ANGÉRIA[1], S. NORGREN[1], T. MATHIESEN[1], M. RISLING[1];
[1]Karolinska Institutet, Stockholm, Sweden; [2]Chalmers Univ. of Technol., Gothenburg, Sweden

Abstract: Background The beneficial effects of N-acetylcysteine (NAC) on CNS ischemia and after TBI in animal models are well documented. However, the bioavailability of NAC is very low. N-acetylcysteine Amide (NACA) is a newly modified form of N-acetylcysteine that contains an amide group in place of the carboxyl group of NAC. NACA has more efficient membrane permeation and crosses the blood brain barrier. We examined the effects of NACA in the secondary inflammatory response following focal penetrating TBI in rats. Material and methods Focal penetrating TBI were produced in a total of 24 male Sprague-Dawley rats randomly selected for treatment (n=5), non-treatment (n=5) and sham (n=4). The treated animals were given NACA 300 mg/kg ip after 5 min and in the 24h survival group a bolus of 300 mg/kg ip after 4h. After 2h and 24h the brains were removed, cut in 14 µm coronal sections and subjected to immunohistochemistry, immunofluorescence, Fluoro-Jade and TUNEL analyses. Results NACA treatment decreased neuronal degeneration by Fluoro-Jade at 24h ($p<.05$). The levels of resident/invading macrophages/microglia in the perilesional area (ox-42) were elevated at 2h and 24h, not differing between groups. The NO-producing inflammatory enzyme iNOS was up-regulated 24h, not differing between groups. Oxidative stress measured by peroxynitrite surrogate marker 3-Nitrotyrosine was detectible at 2h and 24h, not differing between groups. Antioxidative enzyme MnSOD was up regulated at 2h and 24h, expressing higher levels at 24h in the NACA group ($p<.05$). NFkB located in the nuclei was up-regulated at 2h and 24h, not differing between groups. Apoptotic cells by TUNEL was up regulated at 2h and 24h, with decreased levels at 2h in the NACA group ($p<.05$). Total levels of Cytochrome c and Bcl-2 did not differ between groups. Conclusions NACA treatment decreased apoptosis and neuronal degeneration and increased antioxdative enzyme MnSOD. The antiapoptotic effect was not linked to alterations in total levels of Cytochrome c or Bcl-2. Our results suggest that NACA

treatment after focal TBI may be beneficial in preventing brain tissue damage, thus showing potential for clinical implications.

Disclosures: **M. Günther:** None. **J. Davidsson:** None. **S. Plantman:** None. **M. Angéria:** None. **S. Norgren:** None. **T. Mathiesen:** None. **M. Risling:** None.

Nanosymposium

486. Traumatic Brain Injury: Therapeutic Strategies I

Location: 156

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 486.07

Topic: C.10. Trauma

Support: University of Arizona Undergraduate Research Award to Uma Raman

Title: Ultrasound promotes neurite outgrowth - Implications for TBI

Authors: U. RAMAN[1], S. PARKER[2], C. DUFFIELD[3], S. GHOSH[2], *S. R. HAMEROFF[4]; [1]Anesthesiol., [2]Cell. and Mol. Med., [3]Ctr. for Consciousness Studies, Univ. of Arizona, Tucson, AZ; [4]Univ. Arizona Med. Ctr., Tucson, AZ

Abstract: Recovery from traumatic brain injury (TBI) involves neurite outgrowth to re-establish synapses, networks and function. Neurite outgrowth depends on cytoskeletal microtubules, disrupted in TBI. Microtubules (MTs) have resonant electromagnetic oscillations in the megahertz (MHz, millions of Hz) frequency range that may be functional (1-3). Ultrasound (US) consists of MHz pressure waves, i.e. 'sound' above human hearing that propagates through tissue and reflects off surfaces, e.g. in medical imaging. Applied to the scalp, US is attenuated by the skull but reaches the brain, reflecting back sufficiently to image brain cortical surfaces (transcranial ultrasound - 'TUS'). In animals, TUS causes electrophysiological and behavioral changes (4). Along with transcranial magnetic and electrical stimulation (TMS, TDcS), TUS has been used for human brain stimulation. For example low-intensity (non-thermal) TUS applied to scalp and (fronto-temporal) brain of human volunteers at 2 or 8 MHz for 15 or 30 seconds improves mood for 30 to 40 minutes compared to sham exposure (5,6). Ultrasound focused on human somatosensory cortex improves sensory discrimination task performance (7). To investigate neuronal-level US effects possibly relevant to TBI and developmental disorders, we extracted cortical neurons from 18 day embryonic Sprague-Dawley rats and grew them as dissociated cultures. We then exposed the neurons to 90 secs of 2 MHz US (1 volt amplitude sine waves from an Agilent 33120 function generator driving a Stem Inc ultrasonic transducer Model

F3000), or sham exposures (zero amplitude). We then quantified neurite outgrowth by a blinded observer counting the number of neurons with or without neurites at 4, and ~24 hours post-exposure. At 4 hours post-exposure, in 5 of 5 experiments, the percentage of neurons with neurites in the US-treated group was ~15% greater than controls ($p<0.007$ or lower for all trials). By ~24 hours, no difference was seen, suggesting a transient but significant effect of US on neurite outgrowth. As TUS is safe, noninvasive and inexpensive (e.g. portable headsets) our results suggest frequent, low intensity (nonthermal) transcranial ultrasound ('TUS') could benefit mood and cognitive function following TBI and other brain disorders. References: (1) Sahu et al (2013a) Biosens Bioelectron 47:141-8; (2) Sahu et al (2013b) Appl Phys Lett 102:123701 (3) Hameroff & Penrose (2014) Physics of Life Reviews (4) Tufail et al (2010) Neuron 66(5):681e94. (5) Hameroff et al (2013) Brain Stimulation 6:409-415 (6) Sanguinetti et al (2014) in preparation (7) Legon et al (2014) Nature Neuroscience 17:322-329

Disclosures: **U. Raman:** None. **S.R. Hameroff:** None. **S. Parker:** None. **C. Duffield:** None. **S. Ghosh:** None.

Nanosymposium

486. Traumatic Brain Injury: Therapeutic Strategies I

Location: 156

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 486.08

Topic: C.10. Trauma

Support: Funding: Supported by the Department of the Army W81XWH-09-1-0443 to KMS.

Title: Post-blast treatment with Nociceptin/Orphanin FQ peptide receptor antagonist reduces traumatic brain injury-induced cerebral hypoxia

Authors: *H. O. AWWAD[1,2], C. SIMPSON-DURAND[3], L. P. GONZALEZ[4,2], P. TOMPKINS[5], Y. ZHANG[3,2], M. LERNER[6,8], D. J. BRACKETT[6], D. M. SHERRY[7,2], V. AWASTHI[3], K. M. STANDIFER[3,2,7];
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Abstract: The occurrence of mild traumatic brain injury (mTBI) has increased recently due to aggressive sports and blast-related injuries. Cellular mechanisms and pathology of mTBI

generally and blast-induced TBI specifically are not completely understood and the search is ongoing for therapeutic targets and biomarkers for injury prognosis in mTBI. Nociceptin Orphanin/FQ (N/OFQ), an endogenous neuropeptide and its Gi/o-protein coupled receptor, the N/OFQ peptide (NOP) receptor modulate various biological functions in the CNS, including nociceptive sensitivity, stress and anxiety, learning and memory, motor control and cytokine release. Previous reports have indicated that N/OFQ contributes to post-injury ischemia following mechanical brain injury, yet its specific role in cerebral hypoxia, vestibulomotor function and injury marker expression following blast-induced TBI is not known. This study is the first to identify the direct association of N/OFQ and its receptor, NOP with TBI-induced changes following an 80 psi head blast exposure in male rats. Radioimmunoassay studies revealed increased expression of N/OFQ in brain tissue and plasma of rats exposed to a single head blast compared to sham rats. Positron emission tomography (PET) studies using intravenous 18-F-fluoromisonidazole indicated that NOP receptor antagonist treatment also reduced TBI-induced cerebral hypoxia when assessed 8-10 days post-blast. Furthermore, one post-blast treatment with a NOP receptor antagonist improved acute vestibulomotor performance on the rotarod compared to blast-injured rats receiving vehicle treatment. The blast-induced elevation of pro-apoptotic proteins and injury markers in related brain regions was also reduced in rats receiving the NOP receptor antagonist. This study reveals an apparent role for the NOP receptor system in blast TBI and suggests potential therapeutic utility of NOP receptor antagonists for mild TBI.

Disclosures: H.O. Awwad: None. **C. Simpson-Durand:** None. **L.P. Gonzalez:** None. **P. Tompkins:** None. **Y. Zhang:** None. **M. Lerner:** None. **D.J. Brackett:** None. **D.M. Sherry:** None. **V. Awasthi:** None. **K.M. Standifer:** None.

Nanosymposium

486. Traumatic Brain Injury: Therapeutic Strategies I

Location: 156

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 486.09

Topic: C.10. Trauma

Support: NIH NINDS NS072873

Title: Cerium oxide nanoparticles improve functional outcomes following mild traumatic brain injury

Authors: *P. J. VANDEVORD[1], V. S. SAJJA[1], Z. BAILEY[1], K. S. HOCKEY[2], C. THORPE[2], A. S. FREY[2], J. A. BATES[2], C. A. SCHOLAR[2], B. LOCKLER[2], B. RZIGALINSKI[2]; [1]Biomed. Engin., Virginia Tech. Univ., Blacksburg, VA; [2]Virginia Col. of Osteo. Med., Blacksburg, VA

Abstract: Traumatic brain injury (TBI) has one of the costliest impacts on society in terms of morbidity, mortality, and socio-economic issues. TBI affects over 2 million people each year in the United States, and absorbs a substantial volume of annual healthcare costs; not to mention the dramatic reduction in quality of life for individuals exposed to this injury. Further, TBI, including mild TBI (mTBI) such as concussion, are associated with many late-onset neurological disorders which develop years to decades after the initial insult. Despite considerable research efforts in this arena, treatment options remain limited. Free radicals are thought to play a key role in the pathophysiology of TBI and secondary brain injury in particular and the accumulation of free radical damage contributes to poor functional outcomes. Previous work in our laboratories has demonstrated the beneficial effect that cerium oxide nanoparticle (CeONP) administration has following neuronal injury and neurodegeneration. This study was conducted to test the hypothesis that post-injury CeONP administration improves functional outcomes following mTBI. We utilized the rodent model of lateral fluid percussion injury with animals either receiving a mild level pressure pulse (1.5 atm) or sham animals which underwent surgery without receiving the injury. CeONP was administrated in 3 doses at 1 min, 15 min and 3 hrs following the injury. Behavioral assessments (novel object recognition (NOR), open field task (OF) and Morris water maze (MWM)) were conducted for up to two weeks post injury. The functional results depicted a significant improvement in both the short-term memory (NOR) and anxiety-like behavior (OF) ($p<0.04$ when compared to CeONP to sham group). Interestingly, MWM data only depicted a trend in improvements, which suggest MWM may not be sensitive enough for mild injury detection. Molecular analysis demonstrated that treatment with CeONP decreased brain lipid peroxidation and serum isoprostanes in injured animals. Overall, the data suggests that CeONP may provide a mechanism for antioxidant activity and neuroprotection. CeONP may provide a multifunction approach to clinical treatments for TBI which are currently lacking.

Disclosures: P.J. Vandevord: None. **V.S. Sajja:** None. **Z. Bailey:** None. **K.S. Hockey:** None. **C. Thorpe:** None. **A.S. Frey:** None. **J.A. Bates:** None. **C.A. Scholar:** None. **B. Lockler:** None. **B. Rzigalinski:** None.

Nanosymposium

486. Traumatic Brain Injury: Therapeutic Strategies I

Location: 156

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 486.10

Topic: C.10. Trauma

Support: CNRM Grant G170AZ

Title: Early intervention attenuates cerebral vulnerability in repeated mild blast-induced traumatic brain injury

Authors: A. KAMNAKSH[1], F. AHMED[1], G. BAIK[3], S. YANG[3], E. BARRY[2], N. E. GRUNBERG[4], J. B. LONG[5], *D. V. AGOSTON[6];
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Abstract: Mild traumatic brain injuries (mTBIs) or concussions are the most prevalent albeit least understood form of traumatic brain insults. In both military and civilian environments, a significant proportion of individuals who have suffered mTBIs sustain additional insults when they return to duty or play. Despite the mild and transient nature of mTBI symptoms, studies have suggested that repeated mTBIs (rmTBIs) can significantly increase the risk of developing neurodegenerative conditions such as chronic traumatic encephalopathy. The short- and long-term consequences of rmTBIs are significantly worse if subsequent insults take place within the period of increased cerebral vulnerability (ICV). The exact pathomechanisms underlying ICV are not fully known but oxidative stress has been implicated. In searching for an FDA approved compound that can ameliorate such metabolic changes we identified 2-Methoxyestradiol (2ME2), an endogenous estradiol metabolite. 2ME2 has been shown to normalize hypoxia-inducible factor 1α and vascular endothelial growth factor levels, thereby mitigating the effects of hypoxic damage. In our study we aimed to test if acute 2ME2 treatment can mitigate the cerebral vulnerability that follows mild blast-induced TBI (mbTBI) and in turn reduce the cumulative effect of repeated mbTBI. Rats were exposed to mild blast overpressure or anesthetized as sham controls; a subset of each was intraperitoneally treated with 5 mg/kg 2ME2 or DMSO (vehicle) immediately after the first exposure. Injured rats were then exposed to 2 more mild blasts. We measured animals' vitals and open field activity at baseline and a number of post-injury time points. At the conclusion of the experiment (2 weeks post-injury), we tested the effect of 2ME2 on serum biomarkers related to various pathologies. We found that acute 2ME2 treatment prevented the cumulative drop in oxygen saturation levels measured in vehicle-treated rats. 2ME2 attenuated the blast-induced decrease in horizontal activity, an index of general health and movement, 1 day post-injury. However, the treatment did not alleviate the decrease in vertical activity, an index of depression-related behaviors, 1 day post-injury and even

increased such behavior at 2 weeks. Importantly, 2ME2 treatment normalized the serum levels of protein biomarkers related to oxidative stress, vascular pathologies, inflammation, as well as neuronal and glial cell death and/or damage. These preliminary findings warrant further investigation to determine the potential of 2ME2 in attenuating cerebral vulnerability and reducing the risk of developing neurodegenerative conditions after rmTBIs.

Disclosures: **A. Kamnaksh:** None. **F. Ahmed:** None. **G. Baik:** None. **S. Yang:** None. **E. Barry:** None. **N.E. Grunberg:** None. **J.B. Long:** None. **D.V. Agoston:** None.

Nanosymposium

486. Traumatic Brain Injury: Therapeutic Strategies I

Location: 156

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 486.11

Topic: C.10. Trauma

Support: NIH NS038684

Title: 20-hydroxyeicosatetraenoic inhibition attenuates cortical lesion, microglia activation and blood-brain barrier damage in pediatric rat model of traumatic brain injury

Authors: ***S. SHU**[1,2], Z. ZHANG[2], X. LIU[4], M. SARASWATI[3], C. ROBERTSON[5], S. KANNAN[5], R. KOEHLER[6];
[1]Dept. of Anesthesiol., Children's Hospital,Chongqing Med. Univ., Chongqing, China; [2]Dept. of Anesthesiol. and Critical Care Med., [3]Dept. of Anesthesiol. and Critical Care Medicine,Johns Hopkins Univ., Baltimore, MD; [4]Dept. of Anesthesiol. and Critical Care Med., Baltimore, MD; [5]Dept. of Anesthesiol. / Critical Care Med. and Pediatrics, Baltimore, MD; [6]Dept. of Anesthesiology/Critical Care Med., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Previous work has shown that inhibition of 20-hydroxyeicosatetraenoic acid (20-HETE) formation by cytochrome P450 metabolism of arachidonic acid can protect immature and mature brain from ischemia. Because traumatic brain injury (TBI) can share some common mechanisms of neurodegeneration, we explored whether post-treatment with the 20-HETE synthesis inhibitor N-hydroxy-N-(4-butyl-2-methylphenyl) formamidine (HET0016) can protect the immature brain from TBI. Male Sprague Dawley rats (P10) were subjected to controlled cortical impact (CCI; velocity 5.0-5.5 m/s; 3 mm diameter; depth 1.5 mm). Age-matched rats were randomly divided into 5 groups: 1) naïve group, 2) sham-operated group, 3) TBI group, 4) vehicle-treated TBI group, and 5) HET0016-treated TBI group. At 3 days of recovery, lesion

volume in the HET0016 TBI group (6.2±2.1 % of hemisphere; ±SD) was reduced compared to the TBI group (13.2±3.3) and the vehicle TBI group (8.3±0.8) (n=6 per group). Western blots of the endothelial tight junction protein zona occludens-1 (ZO-1), occludin and claudin-5 revealed that HET0016 treatment mitigated the reduced expression seen after TBI. Analysis of morphology of ionized calcium-binding adapter molecule-1 (Iba-1)-stained microglia in the peri-lesion region indicated that microglia in cortex, corpus callosum, hippocampus, striatum, thalamus and piriform cortex were activated. Microglia with more intersections (87.2±15.1) and branches (6.1±1.1) was observed in the HET0016-treated group than in TBI (65.2±12.0, 4.70±0.9, respectively) and vehicle TBI groups (68.2±10.6, 4.9±0.8). With regard to the soma volume and the intersection and length of branches, there was no significant difference between HET0016-treated TBI group and naïve/sham group. In separate cohorts, real time PCR of tissue samples indicated that gene expression of IL-1and TNF- α was increased among the M1 microglia markers (IL-1β, TNF-α, IL-12p35, IL-12p40) in TBI groups. However, the M2 microglia markers (IL-4, IL-10) were greater in the HET0016-treated TBI group than in the TBI and vehicle TBI. To determine whether HET0016 exerts direct modulatory effects on microglia activation, BV2 microglia cells (5×10^{5}) were stimulated with LPS (100 ng) for 3 h, and wells then were treated with HET0016 (20-30 μM) or vehicle for 1 h. LPS stimulation decreased BV-2 cell viability (MTT assay) while treatment with HET0016 was protective. Our data show that 20-HETE contributes to neural injury after TBI in immature brain. The mechanism may involve a more rapid progression of microglia to the M2 adapted phenotype and reduction in the loss of blood-brain barrier tight junction protein expression.

Disclosures: **S. Shu:** None. **Z. Zhang:** None. **X. liu:** None. **M. Saraswati:** None. **C. Robertson:** None. **S. Kannan:** None. **R. Koehler:** None.

Nanosymposium

486. Traumatic Brain Injury: Therapeutic Strategies I

Location: 156

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 486.12

Topic: C.10. Trauma

Support: The Roskamp Foundation

DOD Grant W81XWH-10-1-0759

Title: Microglia polarization dynamics in a mouse model of single and repetitive mtbi

Authors: *B. C. MOUZON[1,2], G. AIT-GHEZALA[1,2], O. OJO[1], C. BACHMEIER[1,2], S. FERGUSON[1,2], M. MULLAN[1], F. CRAWFORD[1,2];
[1]The Roskamp Inst., Sarasota, FL; [2]James A. Haley Veterans Hosp., Tampa, FL

Abstract: The impact of traumatic brain injury (TBI) has been extensively studied in the last decade, and these studies revealed that repeated mild, and moderate to severe TBI causes chronic microglial activation that may be the substrate of various neurodegenerative diseases. Microglia and macrophages are the primary mediators of immune defense in the central nervous system. Microglia and macrophages have 2 major phenotypes, including a classically activated, proinflammatory (M1) state that might contribute to neurotoxicity, and an alternatively activated (M2) state that promotes repair. Although both types of inflammation are thought to have an important role at an acute time point following injury, the dual role of distinctly polarized microglia/macrophage in the pathobiology of traumatic brain injury at acute and chronic time points has not been explored. The overarching aim of this study is to identify the temporal kinetics of microglia/macrophage polarization after mild TBI using our well-established murine model of closed head injury. To date, we have found that animals receiving single injury displayed evident pathology, manifest as neuroinflammation and white matter loss, which peaked at 6 months and remained static at the 12 months evaluation. In contrast, not only did the repetitively injured mice show greater pathological changes at all-time points when compared to the single injury animals, there was also evidence of progression of neuropathology from 6 to 12 months post injury. This study characterize the dynamics of the microglia M1/M2 phenotype following single or multiple injury at acute and chronic time points post injury. To achieve this goal, we are conducting both neuropathological and gene expression analyses of three microglia/macrophage M1/M2a, c phenotypes. Immunofluorescence and gene expression analysis will be performed to detect M1 microglia and macrophage subtype markers: (CD32, CD16, iNOS, CD11b, CD86), M2a wound healing/repair subtype (Ym1, Arg-1, CCL22, TGM2) and M2c regulatory subtype (IL-10, TGF-beta, CCR2) phenotype genes Our goal is to “flank” the critical time period within which we hypothesize the shift from favorable to unfavorable TBI-dependent neuroinflammatory response occurs, i.e. M2 to M1. This work has high translational potential for patients affected by both mild and severe chronic head injury at chronic time points, refining the nature and timing of treatment strategies, and potentially decreasing or delaying the risk of future neurodegenerative disease.

Disclosures: B.C. Mouzon: None. **G. Ait-Ghezala:** None. **O. Ojo:** None. **C. Bachmeier:** None. **S. Ferguson:** None. **F. Crawford:** None. **M. Mullan:** None.

Nanosymposium

486. Traumatic Brain Injury: Therapeutic Strategies I

Location: 156

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 486.13

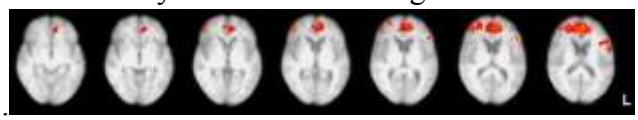
Topic: C.10. Trauma

Support: James S. McDonnell "Scholar Award"

Title: Order from chaos: Return of thalamo-frontal connectivity after severe brain injury

Authors: *M. M. MONTI[1], P. M. VESPA[2];
[2]Neurosurg., [1]UCLA, Los Angeles, CA

Abstract: In the domain of disorders of consciousness, it is well known that the return of awareness after severe brain injury is tied to the restoration of thalamo-frontal connectivity. Although this finding is well accepted, the evidence it relies on is mainly tied to a single case report (Laureys et al., 2000) in which thalamo-frontal connectivity, then measured with PET, appeared re-established after a patient had recovered consciousness. In the present work we address the issue using fMRI in a cohort of traumatic brain injury patients. Specifically, we recruited patients recovered in the intensive care unit at the Ronald Reagan Medical Center at UCLA with moderate-to-severe traumatic brain injury and performed two resting state BOLD fMRI scans. The first took place in the acute setting, generally within 10 days post trauma, while the second took place at approximately six months post injury. In addition to neuroimaging measurements patients were also assessed behaviorally (with the Glasgow Coma Scale -- GCS; and Glasgow Outcome Scale extended -- GOSe). Neural data were analyzed using a seed-based approach. As seed region we employed bilateral thalamus, and assessing the correlation between its spontaneous low-frequency fluctuations and those of all other regions of the brain. Overall, we find that in the acute scan patients exhibited a widespread disruption of brain connectivity organization, mainly observable as a hypo-connectivity between thalamus and medial prefrontal cortex, which appeared restored, at the group level, after 6-months (see Figure 1; consistent with Laureys et al., 2000). However, the return of thalamo-frontal connectivity appeared to be a general pattern (inclusive of patients who were already conscious at the time of the first scan), and is more weakly correlated to changes in behavioral measures across the two time-points.



Disclosures: **M.M. Monti:** None. **P.M. Vespa:** None.

Nanosymposium

486. Traumatic Brain Injury: Therapeutic Strategies I

Location: 156

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 486.14

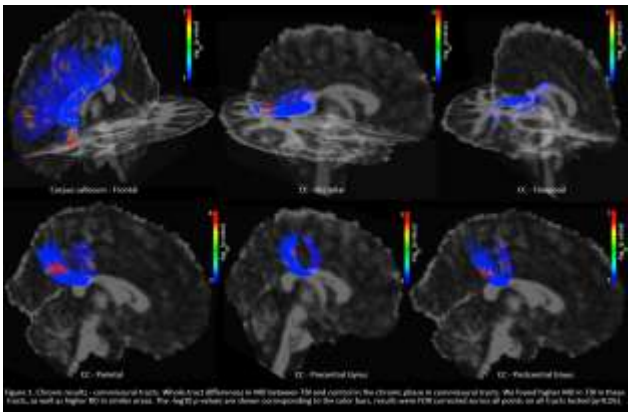
Topic: C.10. Trauma

Support: NIH R01 HD061504

Title: Tract-based analysis of white matter disruption in moderate-to-severe pediatric traumatic brain injury

Authors: *E. L. DENNIS[1], Y. JIN[1], J. VILLALON[1], C. KERNAN[2], R. MINK[4], T. BABIKIAN[2], C. GIZA[3], R. ASARNOW[2], P. THOMPSON[1];
[1]Imaging Genet. Center, INI, LONI, Keck SoM USC, Marina Del Rey, CA; [2]Dept of Psychiatry and Biobehavioral Sciences, Semel Inst. for Neurosci. and Human Behavior, [3]Brain Injury Res. Center, Dept of Neurosurg. and Div. Ped. Neurol., Mattel Children's Hosp., UCLA, Los Angeles, CA; [4]Pediatrics, Harbor UCLA Med. Ctr., Los Angeles, CA

Abstract: Traumatic brain injury (TBI) is the leading cause of death and disability in children and can have many long-lasting consequences. Brain imaging methods such as DTI (diffusion tensor imaging) are especially sensitive to white matter (WM) damage in TBI. However, tractography is complicated by damage and decreased FA (fractional anisotropy) characteristic of TBI, which can result in incomplete tract reconstruction, but we utilize a new method that does not suffer from these issues. Here, we examine pediatric moderate/severe TBI patients longitudinally, scanned in both the postacute (2-4 months post injury) and chronic phase (12 months post injury). We used the newly developed autoMATE (automated multi-atlas tract extraction) to identify differences in WM integrity. We assessed FA, RD (radial diffusivity), MD (mean diffusivity), and AD (axial diffusivity) along 18 different tracts, based on the Eve atlas. For the post-acute phase, we had 70 participants: 35 TBI (mean age=14.0 years, 9 F) and 35 control (mean age=15.0 years, 16 F). We also studied 41 participants in the chronic phase, 35 of whom were participants included in the post-acute phase, and 6 of whom did not have post-acute data: 19 TBI (mean age=16.1 years, 5 F), 22 healthy controls (mean age=16.2 years, 7 F). Postacutely, we found lower FA in the TBI in the frontal CC, mainly in the projections of the body and genu, not the midline. We also found lower FA in TBI in the left IFOF (inferior fronto-occipital fasciculus) and left ILF (inferior longitudinal fasciculus). In the chronic phase, we found greater differences, and these were in the measures RD and MD, rather than FA. We found higher RD and MD in TBI in all CC segments, consistently in the lateral projections from the genu, body, and splenium, and including the forceps major and minor. We also found higher RD and MD in TBI in the bilateral ATR (anterior thalamic radiation), right CST (corticospinal tract) bilateral cingulum, right IFOF, and bilateral ILF. This suggests a distributed pattern of WM disruption that continues over the first year following a traumatic brain injury.



Disclosures: **E.L. Dennis:** None. **Y. Jin:** None. **J. Villalon:** None. **P. Thompson:** None. **C. Kernan:** None. **R. Mink:** None. **T. Babikian:** None. **R. Asarnow:** None. **C. Giza:** None.

Nanosymposium

487. Biomarkers for Psychosis: EEG and Imaging

Location: 150B

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 487.01

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

Support: NCRR: UL1 RR024156

Title: Neural synchrony between the DLPFC and ipsilateral and contralateral cortical regions in schizophrenia patients and healthy controls during a working memory task

Authors: ***R. SO**[1], F. STEFFEN-ALLEN[2], L. S. KEGELES[3], J. J. CHROBAK[1], C.-M. A. CHEN[1];
[1]Psychology, [2]Dept. of Psychology, Univ. of Connecticut, Storrs, CT; [3]Dept. of Psychiatry, Columbia Univ., New York, NY

Abstract: Neural synchrony of gamma oscillations between the dorsolateral prefrontal cortex (DLPFC) and cortical regions has been hypothesized to drive coordinated working memory function. Patients with schizophrenia have been found to have abnormalities in this communication, which may underlie deficits in working memory. The present study aimed to examine differences in communication patterns between the DLPFC and different cortical areas between schizophrenia patients and healthy controls under comparable working memory performance. Scalp EEG recordings were collected for schizophrenia patients (N = 7) and

healthy volunteers (N = 8) during a modified Sternberg working memory task, which had two levels of task difficulty. Each difficulty level contained 64 trials (total = 128 trials). Only correct, artifact-free trials were analyzed, and there were no group differences in the number of correct trials (independent-samples t-test; $t(13) = -0.659$, $p = 0.521$). Instantaneous phases were extracted by Morlet wavelet decomposition on 98 scales from 0.5 Hz to 60 Hz. Phase locking values (PLVs; ranging from 0 (no synchronization) to 1 (perfect synchronization)) was used as an index of synchronization between the left (F3) or right DLPFC (F4) and another cortical area of interest. On the left hemisphere, the modulation of PLVs between F3 and O1 was significantly different between groups, with patients showing greater neural synchrony when task difficulty increased ($t(13) = -2.161$, $p = 0.050$). Patients also showed greater modulation of PLVs between other frontal electrodes surrounding F3 and O1 (AF7/O1: $t(13) = -3.315$, $p = 0.006$; F7/O1: $t(13) = -2.295$, $p = 0.039$; F5/O1: $t(13) = -2.646$, $p = 0.020$; F1/O1: $t(13) = -2.678$, $p = 0.019$, and Fz/O1: $t(13) = -2.644$, $p = 0.020$). On the right hemisphere, patients demonstrated greater modulation of PLVs between F4 and T8 ($t(13) = -2.627$, $p = 0.021$) and maintained the same pattern for other electrodes around F4 and T8 (AF8/T8: $t(13) = -2.485$, $p = 0.027$; AF4/T8: $t(13) = -3.058$, $p = 0.009$, and F2/T8: $t(13) = -3.259$, $p = 0.006$). This difference in neural synchrony patterns may reflect a visual-based compensatory strategy in patients to maintain the same level of working memory performance as controls.

Disclosures: R. So: None. **F. Steffen-Allen:** None. **L.S. Kegeles:** None. **J.J. Chrobak:** None. **C.A. Chen:** None.

Nanosymposium

487. Biomarkers for Psychosis: EEG and Imaging

Location: 150B

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 487.02

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

Support: NIH Grant 5-R31-MH058251-06-10

Conte Grant 5-P50-MH064065-07-10

NARSAD Independent Investigator Award to AB

Title: Neural correlates of aberrant affective and attentional processing in individuals at familial high-risk for schizophrenia

Authors: *E. H. ANDERSEN[1], A. M. CAMPBELL[1], S. E. SCHIPUL[1], F. C. L. DONKERS[2], A. BELGER[1];
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Abstract: Background: Although attentional and affective disturbances are core deficits of schizophrenia (SCZ), the underlying neural mechanisms remain poorly understood. First-degree relatives (FDRs) and prodromal individuals also demonstrate disruptions in attentional processing, suggesting that attentional abnormalities precede psychosis onset and may represent vulnerability markers. Despite the clinical presentation of affective symptoms, SCZ patients show relatively intact valence discrimination and appropriate reports of valence and arousal. Additionally, SCZ patients exhibit widespread dysfunctional neural oscillations, including deficiencies in beta, theta, and delta activity, potentially underlying aberrant attention and affective network connectivity. The goal of the current study was to determine whether FDR individuals demonstrate comparable neural correlates of attentional and affective processing as SCZ patients, and identify novel vulnerability markers using time-amplitude and time-frequency analyses. Method: EEG was recorded during an emotional oddball paradigm to investigate neural mechanisms supporting attentional (P3b) and affective (late positive potential (LPP)) processing disparities in 31 SCZ patients, 28 FDR individuals, and 47 healthy controls. Time-frequency analyses were performed using a wavelet transform to assess total power, evoked power, and intertrial (phase) coherence. Results: FDR participants exhibited aberrant affective processing, evident by the distinct enhancement of LPP amplitude and evoked beta oscillatory activity relative to controls and SCZ patients. Furthermore, FDR participants demonstrated an intermediate reduction in Target-P3b amplitude and evoked power in the delta band compared with controls and SCZ patients. Neural oscillations in delta and theta frequencies and their synchronization were impaired in SCZ patients. Conclusion: FDR participants appear to be employing a compensatory mechanism, evident by the enhanced LPP amplitude and increased beta oscillatory activity, which may represent over-engagement of limbic neural circuitry necessary for FDR participants to perform equivalent to controls behaviorally and remain asymptomatic. FDR participants and SCZ patients shared deviations in attentional processing, indicated by the reduced Target-P3b, and aberrant delta and theta oscillatory activity. Disruptions in delta and theta oscillatory activity may reflect deviations in fronto-temporal and fronto-striate connectivity. The LPP and beta oscillatory activity may be valuable as vulnerability markers in the detection and prediction of psychopathology.

Disclosures: E.H. Andersen: None. **A.M. Campbell:** None. **S.E. Schipul:** None. **A. Belger:** None. **F.C.L. Donkers:** None.

Nanosymposium

487. Biomarkers for Psychosis: EEG and Imaging

Location: 150B

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 487.03

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

Support: VA RR&D D7008W

NARSAD Young Investigator Award, Brain and Behavior Research Foundation

Title: Application of machine learning to identify features of EEG associated with working memory performance in healthy adults and schizophrenia

Authors: *C.-M. A. CHEN[1], R. JIANG[2], J. G. KENNEY[3,4], J. BI[2], J. K. JOHANNESEN[4,3]; [1]Psychology, [2]Computer Sci. and Engin., Univ. of Connecticut, Storrs, CT; [3]VA Connecticut Healthcare Syst., West Haven, CT; [4]Psychiatry, Yale Univ., New Haven, CT

Abstract: With millisecond-level temporal resolution, electroencephalographic (EEG) recording is a powerful tool for studying neural dynamics of human cognition. However, the massive EEG data (i.e., temporal, spatial, spectral measures, and interactions) requires a computational framework to answer experimental questions through integration of multiple features. Machine learning, designed to discover knowledge from big data through modeling, was evaluated in this study as a method to identify EEG features associated with working memory task performance in healthy and schizophrenia samples. Classifiers were built to answer questions concerning what frequency components and stages of information processing are most critical to memory performance and to schizophrenia pathophysiology. A visual Sternberg working memory task (90 trials, span 4-8 items) was administered to healthy normal (HN; n=14) and schizophrenia (SZ; n=40) samples during EEG. Sixty features were extracted based on processing stage (4; pre-stimulus baseline, encoding, retention, retrieval), frequency band (5; delta, theta, alpha, beta, gamma), and electrode (3; Fz, Cz, Oz). One-norm support vector machines (SVM) were used to classify: (1) correct vs. incorrect trial performance within each sample, and (2) HN vs. SZ status across correct and incorrect trials. Model 1 identified frontal (Fz) gamma activity during encoding and occipital (Oz) theta activity during retrieval as primary classifiers of task accuracy in HN. The valence of coefficients indicated increased activity in both regions with higher memory challenge. Model performance statistics were robust (AUC=1.00, accuracy=0.92, recall=1.00) and cross-validation using SZ data yielded acceptable accuracy (0.74). As for HN, Model 1 in SZ identified frontal gamma, in addition to central (Cz) gamma, during encoding as primary classifiers of task accuracy. Overall model performance was acceptable(AUC=0.86,

accuracy=0.79, recall=0.83). Regarding group differences (Model 2), SZ was associated with frontal-central low-frequency activity (delta, theta) during the pre-stimulus baseline on correct trials, while frontal alpha during retrieval was associated with HN performance on incorrect trials. The EEG features identified by machine learning replicate literature reports, including upward modulation of gamma with increased memory load during encoding, increased low-frequency activity in resting EEG of SZ, and involvement of alpha in memory retrieval. Model performance statistics further support the accuracy and stability of results, and utility of machine learning as a data reduction solution for EEG experimentation.

Disclosures: C.A. Chen: None. **R. Jiang:** None. **J.G. Kenney:** None. **J. Bi:** None. **J.K. Johannesen:** None.

Nanosymposium

487. Biomarkers for Psychosis: EEG and Imaging

Location: 150B

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 487.04

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

Support: NIH R01 MH58251

Title: Neural connectivity of executive and affective networks in schizophrenia patients and relatives

Authors: *A. M. CAMPBELL, S. E. SCHIPUL, A. BELGER;
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Abstract: Background: Schizophrenia has been associated with disordered brain connectivity, as revealed by both fMRI (e.g., Lynall et al., 2010) and EEG (e.g., Donkers et al., 2011). Executive and affective processing are core deficits of the disorder, precede illness onset, and are associated with distinct but highly interconnected neural networks. Disruptions in communication within and between these networks may underlie behavioral deficits. Objectives: The goal of this study is to examine brain connectivity within and between executive and affective networks in patients with schizophrenia relative to neurotypical participants, and in first-degree relatives, to study intermediate phenotypic effects. Furthermore, we will integrate fMRI and EEG measures of connectivity, as they reveal unique insights into neural patterns at distinct timescales. Methods: Participants include 15 adults with schizophrenia, 9 first-degree relatives, and 16 neurotypical participants matched for age and gender. (Additional relative and neurotypical participants will

be included in future analyses.) All participants completed separate fMRI and EEG sessions, which included a 1-back task with positive, aversive, and neutral images. Neural synchrony was assessed with time-frequency analyses of EEG data using a complex Morlet wavelet transform. Functional connectivity was assessed with correlation analyses of the fMRI activation timecourse in distinct regions of interest, organized into networks. Results: Both neural synchrony and functional connectivity analyses revealed aberrant connectivity patterns in patients with schizophrenia and relatives. Neural synchrony analyses revealed a group by emotional valence interaction in theta and beta evoked power ($p=.02$; $p=.04$), and in theta inter-trial coherence ($p=.01$) whereby patients show reduced theta synchrony to negative targets compared to controls. Functional connectivity analyses revealed weaker connectivity in participants with schizophrenia in frontal: frontal ($p=.05$) and frontal: cingulate ($p=.04$) executive networks, particularly during trials with aversive stimuli. Relatives also showed moderate disruptions in connectivity relative to neurotypical participants ($p<.06$). Associations were also found between executive network functional connectivity and evoked and total power in theta and beta ($p<.05$), providing converging evidence across distinct methodologies. Conclusions: These findings provide evidence of aberrant brain connectivity in schizophrenia and first-degree relatives in executive and affective networks, which may underlie observed behavioral dysfunction in these domains.

Disclosures: A.M. Campbell: None. **S.E. Schipul:** None. **A. Belger:** None.

Nanosymposium

487. Biomarkers for Psychosis: EEG and Imaging

Location: 150B

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 487.05

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

Support: NIH

Title: Molecular signature in olfactory neuronal epithelium that is correlated with cognitive impairment in schizophrenia

Authors: *Y. HORIUCHI[1,2], Y. TAKAYANAGI[2], T. HO[2], K. TAJINDA[2], N. G. CASCELLA[2], D. SCHRETLEN[2], J. PEVSNER[2], A. SAWA[2];
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Abstract: We explored molecular signature in olfactory neuronal epithelium that is correlated with cognitive impairment in schizophrenia. Olfactory neuronal epithelium was chosen as a study material because it is accessible neural tissue without major invasion. To address this question, patients with schizophrenia (SZ) as well as age- and gender-matched controls were recruited. We performed nasal biopsy followed by laser-captured microdissection to enrich neuronal population (Tajinda et al, Mol Psychiatry, 2010). All the participants also completed a battery of neuropsychological tests. From the neural tissue, we conducted microarray to compare molecular expression profiles between SZ and controls. Then, we studied possible correlation between gene expression and cognitive function for the genes that were differentially expressed in SZ compared with normal controls. Among such genes, we underscored significant correlation between the expression level of SMAD family member 5 (SMAD5) and cognitive function. Furthermore, functional pathway analysis of microarray data revealed that expression levels of multiple genes in the SMAD pathway were altered in SZ, which was further confirmed by quantitative RT-PCR. This exploratory study indicates the importance of further investigation on the SMAD pathway in conjunction with cognitive deficit of SZ.

Disclosures: Y. Horiuchi: None. **Y. Takayanagi:** None. **T. Ho:** None. **K. Tajinda:** A. Employment/Salary (full or part-time):; Astellas. **N.G. Cascella:** None. **D. Schretlen:** None. **J. Pevsner:** None. **A. Sawa:** 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.; Astellas.

Nanosymposium

487. Biomarkers for Psychosis: EEG and Imaging

Location: 150B

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 487.06

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

Support: Stanley Center for Psychiatric Research, The Broad Institute of Harvard and MIT

Title: Common SNP heritability of brain structures and intracranial volume using MRI

Authors: *R. SHAFEE[1,2], A. J. HOLMES[3], G. GENOVESE[2], P. H. LEE[4], L. GERMINE[4], J. L. ROFFMAN[5], J. W. SMOLLER[4], R. L. BUCKNER[7,6,5], S. A. MCCARROLL[1,2];
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Abstract: Brain anatomy is highly heritable as shown by twin studies. With the availability of genotype data it is now possible to estimate the heritability of a disease or a complex trait from single nucleotide polymorphisms (SNPs) of unrelated individuals. The genotype data can be used to determine the level of genetic relatedness between unrelated individuals, which can be used to estimate the fraction of phenotypic variance of a trait that can be explained by genotypic variance. In this work we estimate the heritability of intracranial volume (ICV) and brain subcortical structures utilizing structural MRI and genotype data of 1035 unrelated 18-35 year old individuals. We investigated the heritability of the following subcortical structure volumes: cerebral white matter and gray matter, cerebellar white matter and gray matter, thalamus, putamen, pallidum, caudate nucleus, amygdala, hippocampus and nucleus accumbens. Using 620,000 SNPs with minor allele frequency > 0.01 we estimated the fraction of the phenotypic variance explained by the combined variance arising from these SNPs (h_g^2) using GCTA. We found statistically significant SNP heritability for ICV, left caudate and right caudate volumes with estimated h_g^2 values (95% CI) of 0.35-1, 0.37-1, 0.33-1, respectively. To understand the heritability contribution of different functional categories of SNPs, we categorized the genotyped SNPs as protein-altering (coding) and noncoding and calculated the heritability from each of these categories separately as well as in a joint model. Although only 2.6% of the SNPs were categorized as coding, an average of 20% of the SNP heritability of ICV, left caudate and right caudate volume was attributed to this category. However, this enrichment in heritability at coding SNPs was not statistically significant possibly due to the small sample size.

Disclosures: R. Shafee: None. **A.J. Holmes:** None. **G. Genovese:** None. **J.L. Roffman:** None. **J.W. Smoller:** None. **R.L. Buckner:** None. **S.A. McCarroll:** None. **P.H. Lee:** None. **L. Germine:** None.

Nanosymposium

487. Biomarkers for Psychosis: EEG and Imaging

Location: 150B

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 487.07

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

Support: 1R01MH094524-01A1 (NIMH, Calhoun & Turner)

Title: Reality distortion symptoms correlate with source based morphometry patterns in schizophrenia

Authors: C. N. GUPTA[1], V. D. CALHOUN[1], J. LIU[1], R. L. GOLLUB[2], S. R. SPONHEIM[3], S. EHRLICH[4], *J. A. TURNER[5];
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Abstract: Background: The Scale for Assessment of Positive Symptoms (SAPS) is a widely used tool for measurement of positive symptoms in Schizophrenia, while Source based morphometry (SBM) is a multivariate extension of VBM utilizing independent component analysis to obtain patterns of common grey matter concentration (GMC) variation between two populations. Previous studies have found repeatable GMC patterns which distinguish patients with schizophrenia from their healthy counterparts. We now look at the relationship between specific positive symptoms' severity and GMC loss in these patterns. Methods. We investigated the correlation of two SAP subscales, namely reality distortion and disorganized symptoms, with SBM patterns showing group difference from the Mind Clinical Imaging Consortium (MCIC) dataset, having 124 healthy controls (HC) and 110 patients with schizophrenia (Sz). The SBM module of the GIFT Toolbox (http://mialab.mrn.org/software/gift/) was used to perform the decomposition on the entire dataset with component number being 30. Of those components, 3 components showing group difference were tested in the schizophrenia sample for a relationship with the positive symptom scores. Spearman's correlation was used for both symptom scales as the distributions were not normal. Results. We observed that the SBM component covering Superior Frontal Gyrus and Middle Frontal Gyrus in Sz participants was positively correlated with reality distortion symptoms ($r=0.20, p=0.045$). There was no significant association with disorganized symptom scores. Conclusions. Our results affirm the association reported between positive symptoms like reality distortion and disorganization with GMC reduction in few Sz studies, thereby warranting further research in that direction.

Disclosures: C.N. Gupta: None. **V.D. Calhoun:** None. **J. Liu:** None. **R.L. Gollub:** None. **S.R. Sponheim:** None. **S. Ehrlich:** None. **J.A. Turner:** None.

Nanosymposium

487. Biomarkers for Psychosis: EEG and Imaging

Location: 150B

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 487.08

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

Title: Processing uncertain dynamic context in schizophrenia and delusions

Authors: *H. TAN[1], C. KAPLAN[1], W. HOCKEIMER[1], J. MOLINA[1], J. APUD[2], D. R. WEINBERGER[1];
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Abstract: Introduction: Real world information is often abstract, dynamic and imprecise. Deciding if information represents random fluctuations, or alterations in underlying context engages higher cognitive functions. Dysfunction may contribute to erroneous, rigidly held beliefs in delusions in psychotic disorders. Here we examined cortical-subcortical circuitry engaging anterior and dorsolateral prefrontal cortex at context inference and at feedback learning as subjects processed noisy symbolic information. We examined the implications for delusions in schizophrenia, in particular the hypothesis that disease-related deficiencies in prefrontal cortical decisions about ambiguous context may subsequently be associated with exaggerated subcortical feedback salience. Methods: 24 normal controls (NC) and 17 schizophrenia patients (SZ) performed an event-related fMRI task in a 3T-MRI scanner. Subjects were presented with numerical information varying stochastically about an underlying integer, which occasionally shifted up or down by one unit. Subjects were to respond when they believed the underlying numerical context had changed. Dynamic Causal Models were used to investigate how prefrontal, parietal and midbrain circuitry interacted during context inference (C) and implicit feedback (F) task phases. Results: Schizophrenia patients and controls responded with similar reaction times and received similar feedback, though overall, patients did relatively worse in minimizing prediction error as they made inferences about context. APFC, DLPFC, parietal and subcortical regions were engaged as subjects inferred context change, as well as when they subsequently evaluated implicit feedback about their predictions. Processing information about stable context engaged these regions less. In deciding about context change (C), patients engaged APFC relatively less than healthy controls, in part driven by reduced effective connectivity from DLPFC to APFC. In processing subsequent data indicating reduced uncertainty of their predictions (implicit feedback, F), patients engaged relatively increased mid-brain activation, driven in part by increased DLPFC to midbrain connectivity. These dissociable prefrontal-parietal and subcortical circuit functions were also accentuated in relation to delusions in patients. Conclusions: Patients make inferences about ambiguous information with reduced anterior frontal engagement that is then apparently reinforced inappropriately. This conceivably sets up a vulnerable cascade of aberrant information processing in which delusions may emerge.

Disclosures: H. Tan: None. **C. Kaplan:** None. **W. Hockeimer:** None. **J. Molina:** None. **J. Apud:** None. **D.R. Weinberger:** None.

Nanosymposium

487. Biomarkers for Psychosis: EEG and Imaging

Location: 150B

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 487.09

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

Support: NIH Grant P50MH094268

Stanley Medical Research Institute

NARSAD

Title: Molecular and cellular signature of neuronal cells affected by genetic and environmental factors of major mental illness

Authors: *E. PASSERI[1], A. M. WILSON[1], R. SRIVASTAVA[1], S. SENGUPTA[1], C. BORDON[2], M. A. KONDO[1], M. KOGA[1], A. ANVARI[3], P. A. GOCHMAN[3], C. OBI[4], D. VALLE[4], K. ISHIZUKA[1], J. L. RAPOPORT[3], L. V. JONES-BRANDO[2], R. H. YOLKEN[2], S.-I. KANO[1], A. SAWA[1];
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Abstract: It is difficult to address molecular and cellular signature associated with neuropsychiatric illnesses, because living brain tissue cannot be accessed directly in almost all cases. To overcome this dilemma, the use of surrogate tissues (e.g., olfactory neuronal cells) and the stem cell/cell conversion approach have recently been developed. Here we use the technique of direct conversion from fibroblasts to neuronal cells to address this question. Nonetheless, the low conversion rate of fibroblasts to induced neuronal cells (iN cells) remains a major barrier to using this methodology. In this study, we first tried to improve the conversion rate: the combined use of valproic acid (VPA) and a metabolite of prostaglandin (unoprostone isopropyl) markedly increased the conversion rate. Based on this technical improvement, we addressed the question of how psychiatric illness-associated genetic and environmental risk factors may affect molecular and cellular signature. We used cells from the subjects carrying two representative copy number variants (CNVs) associated with mental illnesses (e.g., 16p11.2 duplication and the 22q11 deletion) as well as cells from normal controls. We observed that these two independent chromosomal changes affect the conversion rate to iN cells and the neurite growth after conversion in a similar manner. Furthermore, we are also testing the effect of Toxoplasma gondii

(T.gondii) exposure, an environmental risk factor for schizophrenia and bipolar disorder. The overall effects of genetic and environmental risk factors to neural phenotypes will be reported.

Disclosures: **E. Passeri:** None. **A.M. Wilson:** None. **R. Srivastava:** None. **S. Sengupta:** None. **C. Bordon:** None. **M.A. Kondo:** None. **M. Koga:** None. **A. Anvari:** None. **P.A. Gochman:** None. **C. Obi:** None. **D. Valle:** None. **K. Ishizuka:** None. **J.L. Rapoport:** None. **L.V. Jones-Brando:** None. **R.H. Yolken:** None. **S. Kano:** None. **A. Sawa:** 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.; Sucampo AG.

Nanosymposium

487. Biomarkers for Psychosis: EEG and Imaging

Location: 150B

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 487.10

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

Support: NIH

Title: Upregulation of miR-124 as a potential molecular signature for schizophrenia and bipolar disorder

Authors: *H. YUKITAKE[1,2], S.-I. KANO[1], K. YAMANAKA[2], F. GOES[1], N. G. CASCELLA[3], J. M. COUGHLIN[1], C. HIGGS[1], J. A. EDWARDS[1], P. K. KIM[1], Y. CHUNG[1], S. NARAYAN[1], H. KIMURA[2], K. HIRAI[2], K. ISHIZUKA[1], A. SAWA[1];
[1]The Johns Hopkins Univ., Baltimore, MD; [2]Takeda Pharmaceut. Co., Fujisawa, Japan; [3]Sheppard Enoch Pratt Hosp., Baltimore, MD

Abstract: We have used olfactory neuronal cells enriched from the nasal biopsy as surrogate tissues that reflect, at least in part, molecular signatures relevant to the brain. We initially conducted microarray and explored pathways that are significantly altered in olfactory cells from patients with schizophrenia (SZ) compared with those from normal controls. Functional group analysis suggested a significant change in the pathway associated with miR-124. Thus, we have tested expression of various microRNAs, including miR-124 and those that have been reportedly associated with SZ. Among them, the most significant change (upregulation) in the expression was confirmed in miR-124, even to greater extent compared with miR-132 and miR-137. We next tested disease specificity, and examined the expression in cells from patients with bipolar

disorder (BP). We again observed significant and even greater extent of augmentation in the expression of miR-124 in BP cells compared with control and SZ cells. We will also report which specific isoform of miR-124 (124-1, -2, and -3) contributes to the upregulation by studying the expression ratio of pri-miR-124-1, -2, and -3. Finally, we will also report how the over-representation of miR-124 results in behavioral changes in animals.

Disclosures: **H. Yukitake:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company. **S. Kano:** None. **K. Yamanaka:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company. **F. Goes:** None. **N.G. Cascella:** None. **J.M. Coughlin:** None. **C. Higgs:** None. **J.A. Edwards:** None. **P.K. Kim:** None. **Y. Chung:** None. **S. Narayan:** None. **H. Kimura:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company. **K. Hirai:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company. **K. Ishizuka:** None. **A. Sawa:** 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.; Collaboration with Takeda Pharmaceutical Company.

Nanosymposium

487. Biomarkers for Psychosis: EEG and Imaging

Location: 150B

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 487.11

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

Support: NIH Grant MH187216

NIH Grant MH274399

Title: Modulation of distributed neural synchrony across the schizophrenia-bipolar spectrum

Authors: *M. E. HUDGENS-HANEY[1], J. B. KNIGHT[1], L. E. ETHRIDGE[2], J. E. MCDOWELL[1], J. A. SWEENEY[2], B. A. CLEMENTZ[1];
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Abstract: Individuals with schizophrenia (SZ) and bipolar disorder (BP) show poor performance on executive and cognitive control tasks. This may be explained in part by a dysfunction in the coordination of distributed neural activity. The current study examined synchronization of neural responses in healthy individuals (N=59) and individuals with schizophrenia (SZ; N=43) and

bipolar disorder (BP; N=55). Participants completed blocked pro- and anti-saccade tasks while electroencephalography (EEG) data was gathered on a 64 channel NeuroScan system. Trials consisted of checkerboards in central and both peripheral visual fields, followed by brightening of one peripheral checkerboard (cue) after 5sec. The central checkerboard flickered at 15Hz. The degree of ssVEP (15Hz) synchronization between the 2016 sensor pairs was assessed and compared using intersensor phase coherence (ISC). ISC values were corrected for chance based on the number of trials for each participant on each task. Those values were averaged for 1000ms epochs and Fisher Z transformed for each participant, condition, and epoch. Task-related changes for each epoch and sensor pair were computed as a function of group and condition (2-way ANOVA). Main effects of group will be discussed. ISC values were considered significant at $p<.005$, with subsequent t-tests using the same threshold. In the 2sec preceding the cue in Anti-saccade trials, controls show greater ISC than both SZ and BP in both short- and long-range connections in and between a number of brain regions. This is consistent with other studies showing that SZ and BP do not properly modulate attention away from the peripheral stimuli during anti-saccade tasks. During the same time in Pro-saccade trials, both SZ and BP show greater ISC than controls between frontal, parietal, and occipital regions. This suggests that SZ and BP are allocating an abnormally large amount of neural activity to continued processing of the central stimulus when it is not relevant to the task. Together, these results indicate that SZ and BP are not sufficiently modulating synchronized neural activity before the cue in either task, despite showing normal behavioral performance on the pro-saccade task.

Disclosures: M.E. Hudgens-Haney: None. **J.B. Knight:** None. **J.E. McDowell:** None. **B.A. Clementz:** None. **L.E. Ethridge:** None. **J.A. Sweeney:** None.

Nanosymposium

487. Biomarkers for Psychosis: EEG and Imaging

Location: 150B

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 487.12

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

Support: Netherlands Organization for Scientific Research (NWO) VIDI Grant 917-46-370

Utrecht University High Potentials Grant

Title: GABA, glutamate and intellectual ability in health and schizophrenia: A 7T 1H-MRS study

Authors: *A. MARSMAN[1], R. C. W. MANDL[1], D. W. J. KLOMP[2], M. M. BOHLKEN[1], V. O. BOER[2], A. ANDREYCHENKO[3], W. CAHN[1], R. S. KAHN[1], P. R. LUIJTEN[2], H. E. HULSHOFF POL[1];
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Abstract: Schizophrenia is characterized by a loss of brain tissue, which may represent an ongoing pathophysiological process. Mechanisms that may be involved are the glutamatergic and GABAergic systems. In this study, levels of GABA (gamma-aminobutyric acid) and glutamate (Glu) levels were examined in 17 patients with schizophrenia (age 28±6, F=4) and 23 matched healthy control subjects (age 28±5, F=7) using proton magnetic resonance spectroscopy (1H-MRS) at a field strength of 7 tesla. Also, correlations between metabolite levels and intelligence measures were investigated. Patients had lower prefrontal GABA levels as compared to healthy controls ($p=0.0012$). Also, there was a significant negative correlation between total intelligence quotient (IQ) and prefrontal GABA levels in patients, with more intelligent patients having lower GABA levels ($p<0.001$). Considering the relatively young age of the sample, this may suggest a role for GABA in the earlier stages of schizophrenia. There was no significant main effect of group on occipital GABA levels and there were no significant main effects of group on Glu levels. In healthy controls, a higher Working Memory Index (WMI) was correlated with significantly lower Glu levels ($p<0.004$) and with higher (but not significantly) GABA levels ($p=0.19$), resulting in a significantly higher GABA/Glu ratio in the occipital cortex ($p=0.04$). In the prefrontal cortex, a higher WMI was correlated with significantly lower GABA/Glu ratios ($p=0.001$). This may suggest that in health, a higher working memory performance is associated with more inhibition in the occipital cortex and more excitation in the prefrontal cortex.

Disclosures: A. Marsman: None. **R.C.W. Mandl:** None. **D.W.J. Klomp:** None. **M.M. Bohlken:** None. **V.O. Boer:** None. **A. Andreychenko:** None. **W. Cahn:** None. **R.S. Kahn:** None. **P.R. Luijten:** None. **H.E. Hulshoff Pol:** None.

Nanosymposium

487. Biomarkers for Psychosis: EEG and Imaging

Location: 150B

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 487.13

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

Support: NIMH

HHMI

NARSAD

Pamlab

Title: Altered error-related activation during working memory performance in schizophrenia: An event-related fMRI study

Authors: *H. ERYILMAZ[1], A. S. TANNER[1], N. HO[1], N. J. SILVERSTEIN[1], D. C. GOFF[2], D. S. MANOACH[1], J. L. ROFFMAN[1];
[1]Psychiatry, Massachusetts Gen. Hosp., Charlestown, MA; [2]Psychiatry, New York Univ., New York, NY

Abstract: Working memory deficits have been reported in schizophrenia and generally been associated with blunted DLPFC activity in patients at increased task loads. However, performance differences between groups can confound interpretation of between-group differences. Here, we acquired fMRI data in 40 schizophrenia (SZ) patients and 40 matched healthy controls using a version of the Sternberg Item Recognition Paradigm, which requires use of working memory function at four different task loads. Using an event-related design, we separately analyzed epochs occurring during correct and incorrect performance. During error epochs, deactivation of the medial prefrontal cortex (mPFC), a critical node in the default network, was stronger in controls than in patients; however, patients who performed more accurately demonstrated stronger error-related mPFC deactivation. Marked between-group differences were also observed in error-related activation throughout the right frontoparietal control network, with controls showing stronger activation than patients. Our results suggest that accurate performance in SZ patients is associated with error-related deactivation in mPFC and highlight the importance of analyzing correct and error epochs separately when assessing between-group differences in working memory tasks.

Disclosures: H. Eryilmaz: None. **A.S. Tanner:** None. **N. Ho:** None. **N.J. Silverstein:** None. **D.C. Goff:** None. **D.S. Manoach:** None. **J.L. Roffman:** None.

Nanosymposium

488. Auditory System: Circuits and Perception

Location: 143A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 488.01

Topic: D.02. Auditory

Title: Encoding of loudness in the auditory brainstem: Responses to a synthetic vowel

Authors: *B. P. HEFFERNAN[1], C. GIGUERE[2], H. DAJANI[1];
[2]Sch. of Rehabil. Sci., [1]Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Objective: The purpose of this study is to expand our understanding of how the human auditory brainstem (ABR) encodes the loudness of speech. **Methods:** Auditory evoked potentials measuring activity from the brainstem of 14 normal hearing subjects (all thresholds <20dB HL from 250-4000Hz; 6 females, 19-45 years of age) were recorded in response to a synthetic 300ms /a/ vowel stimulus (fundamental frequency of 100Hz) presented at four different intensities (55dBA, 65dBA, 75dBA, and 85dBA). Responses were compared across stimuli with respect to their temporal and spectral content. **Results:** Brainstem response latencies changed in a predictable manner in response to changes in intensity, with greater intensity resulting in shorter latency in the encoding of the speech ABR. Spectral analyses of the responses show a good stimulus-response correlation with a low-pass filtered version of the stimulus, indicative of phase locking to the fundamental frequency and to the harmonics of the first two formants of the vowel stimulus. Characteristic changes in the distribution of power at the fundamental frequency and its harmonics were observed with changes to stimulus intensity. Unexpectedly, the amplitude of F0 in the brainstem is not monotonic increasing with increasing intensity, whereas H2 (200Hz) is. The average amplitude of the harmonics about the first formant (centered at H7=700Hz) is also monotonic increasing with increasing intensity of the stimulus. **Conclusions:** To the best of our knowledge, very little is currently known about the brainstem encoding of speech intensity; these data therefore mark the beginning of a comprehensive delineation of how the human auditory brainstem encodes speech intensity. Characterising the encoding of intensity at the brainstem level is important in understanding the perception of loudness, and may provide insight into the perceptual challenges encountered by persons with a hearing impairment.

Disclosures: B.P. Heffernan: None. **C. Giguere:** None. **H. Dajani:** None.

Nanosymposium

488. Auditory System: Circuits and Perception

Location: 143A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 488.02

Topic: D.02. Auditory

Support: NWO Rubicon grant 446-12-010

NIH grant P41 EB015894

NIH grant P30 NS076408

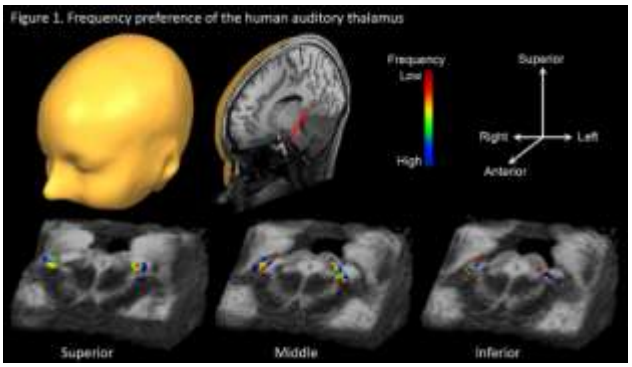
NIH grant S10 RR26783

WM KECK Foundation

Title: Ultra-high field functional MRI reveals tonotopic gradients in the human auditory thalamus

Authors: *M. MOEREL[1], E. FORMISANO[2], R. GOEBEL[2], K. UGURBIL[1], E. YACOUB[1], F. DE MARTINO[2];
[1]Ctr. for Magnetic Resonance Res., Univ. of Minnesota, Minneapolis, MN; [2]Dept. of Cognitive Neuroscience, Fac. of Psychology and Neurosci., Maastricht Univ., Maastricht, Netherlands

Abstract: The medial geniculate body (MGB) of the thalamus is a pivotal relay in the auditory pathway. Due to its small size (4x5x4.5mm in humans), the functional organization of the human MGB remains elusive. Here we use 7T MRI to localize the MGB and explore its processing of frequency and sound location. Anatomical localization of MGB was based on high-resolution short inversion time T1 data (siT1; 0.6 mm isotropic) [1]. Functional MRI time series (1.1 mm isotropic) were collected while subjects (n = 6) listened to binaural recordings of 84 natural sounds (e.g. speech, music), presented at one of seven frontal azimuthal locations (-90 to +90 degrees). The MGB response was modeled as a linear combination of the sounds' features, representing the inseparable coding of frequency and location (6 frequency bins x 7 locations). Individual maps were obtained by color-coding voxels according to frequency and location to which it responded best. Group maps were obtained as the average across 6 individuals after aligning the subjects based on their siT1-defined MGB. Two low frequency regions were identified at the superior-posterior-medial end and inferior-anterior-lateral end of the MGB (see Figure 1). Between these low frequency clusters, voxels were tuned to higher frequencies resulting in a low-high-low tonotopic gradient. While the inferior-anterior-lateral part of the MGB was mostly tuned to contralateral sound locations, in the superior-posterior-medial part of the MGB an approximately equal number of voxels tuned to ipsi- and contralateral space. We interpret the antero-inferior end of the low-high-low tonotopic gradient, tuned to contralateral sound locations, as the lemniscal ventral division of the MGB [2]. The postero-superior part suggests an additional auditory field, reflecting medial or dorsal MGB, or the lateral part of the posterior thalamic nucleus (Pol) [3]. 1. Tourdias, T., et al. (2014). Neuroimage, 84, 534-545. 2. Aitkin, L.M., & Webster, W.R. (1972). J. Neurophysiol., 35, 365-380. 3. Anderson, L.A., & Linden, J.F. (2011). Hear. Res., 274, 48-60.



Disclosures: **M. Moerel:** None. **E. Formisano:** None. **R. Goebel:** None. **K. Ugurbil:** None. **E. Yacoub:** None. **F. De Martino:** None.

Nanosymposium

488. Auditory System: Circuits and Perception

Location: 143A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 488.03

Topic: D.02. Auditory

Support: The Royal Society

HFSP

EMBO

Wellcome Trust

Title: Novel head-fixed behavior, optogenetic and imaging approaches for studying the function of inhibitory interneurons in the auditory cortex of the mouse

Authors: ***D. L. SOSULSKI**, J. C. H. COTTAM, S. AHILAN, M. HAUSSER;
Univ. Col. London, London, United Kingdom

Abstract: One of the central challenges in neuroscience is to elucidate how perceptions, decisions and behaviors arise from patterns of neural activity. Crucial to this is the use of model systems that permit the integrated application of imaging, electrophysiological and optogenetic approaches in behaving animals so we can determine how the cellular components and activity observed in neural circuits ultimately underlie these perceptions and responses. To this end, we

have developed a novel tone discrimination task based on a “lick/no lick” paradigm that can be learned quickly (2-3 weeks) and performed at high levels (> 70% accuracy) by head-fixed mice. In this task, mice learn to discriminate multiple pairs of pure tones, ranging from relatively easy (tones 1/2 of an octave apart in frequency) to quite difficult for a mouse to discriminate (tones only $1/8^{th}$ of an octave apart), and the stimuli in these pairs are presented pseudorandomly over the course of a single behavioral session. In addition, to determine the roles that different classes of inhibitory interneurons play in the performance of this task, we are using Channelrhodopsin-2 and ArchT, in combination with transgenic mouse lines that allow us to selectively express these optogenetic tools in either Parvalbumin-positive (PV+) or Somatostatin-positive (SOM+) inhibitory interneurons in the auditory cortex, to increase or decrease the activity of these cells while animals perform our task. Our preliminary results suggest that decreasing the activity of PV+ interneurons in A1 enhances the ability of mice to discriminate tones, while increasing the activity of PV+ interneurons has no effect on discrimination; furthermore, decreasing the activity of SOM+ interneurons has no effect on discrimination performance, but increasing the activity of SOM+ interneurons leads to a decrease in discrimination performance. Finally, we are also performing two-photon calcium imaging of excitatory and inhibitory neurons in auditory cortex during the learning and performance of this task using GCaMP6S in combination with a chronically implanted glass window to image the same population of cells over the course of several weeks. This combination of approaches is expected to yield new insights into the role these cells play in auditory perception, learning and behavior. Funding provided by: The Royal Society, EMBO, HFSP, Wellcome Trust

Disclosures: D.L. Sosulski: None. **J.C.H. Cottam:** None. **S. Ahilan:** None. **M. Hausser:** None.

Nanosymposium

488. Auditory System: Circuits and Perception

Location: 143A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 488.04

Topic: D.02. Auditory

Support: NSF Grant 140081

Title: Spike-timing precision encodes sound envelope shape in ventral auditory cortical fields

Authors: *C. M. LEE[1], A. OSMAN[2], M. A. ESCABI[2,3], H. L. READ[1,2];
[1]Psychology, [2]Biomed. Engin., [3]Electrical and Computer Engin., Univ. of Connecticut, Storrs, CT

Abstract: Virtually all animals use time-varying (temporal) cues to categorize sounds, communicate and act appropriately within their environments. In mammals, the auditory cortices are essential for behavioral discrimination of temporal cues and yet the neural mechanisms underlying this ability remain unknown. Primary (A1) and ventral non-primary auditory cortical fields are physiologically and anatomically organized and specialized to represent distinct spectral and spatial cues in sound. The current study investigates cortical field differences for encoding envelope shape and rhythm temporal cues in sound. We use shuffled correlation analysis to quantify reliability and precision (jitter) (Joris et al., 2006; Zheng and Escabi, 2008) of single neuron spike timing responses to periodic noise sequences with variations in shape and rhythm. We find spike timing reliability of steady-state responses decreases logarithmically with noise repetition rate for individual neurons and across the population in A1 and ventral cortical fields. Across stimulus conditions, spike timing reliability during the first cycle was greater for A1 than ventral fields. In contrast, steady state responses were more reliable in ventral non-primary fields. A1 responses are primarily sound onset driven with low spike timing jitter indicating more precise temporal cue encoding than ventral fields. In contrast, ventral fields had sustained responses to repetitive noise trains. Furthermore in ventral fields, jitter varies systematically with changes of the envelope shape at low repetition rates. Across regional neuron populations, average spike timing jitter is rank ordered A1 < VAF < cSRAF. These differences suggest a functional hierarchy whereby later developing ventral auditory cortical fields encode sound shape with spike timing jitter, and respond reliably over a reduced range of rhythms, possibly due to slower temporal integration times. This could serve to better encode sound shape cues important for perception of attack and timbre and used to discriminate and categorize sound objects.

Disclosures: C.M. Lee: None. **A. Osman:** None. **M.A. Escabi:** None. **H.L. Read:** None.

Nanosymposium

488. Auditory System: Circuits and Perception

Location: 143A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 488.05

Topic: D.02. Auditory

Support: Rothberg Research Award in Human Brain Imaging

Title: Separate networks for music processing and auditory scene analysis

Authors: *A. S. GREENBERG[1], R. RANDALL[2];
[1]Dept. of Psychology, Univ. of Wisconsin-Milwaukee, Milwaukee, WI; [2]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Musicality can be thought of as a property of sound that emerges when specific organizational parameters are present. Previous work has identified properties of musical repertoires in an attempt to explain particular musical behaviors (e.g. musical expectations) and their neural correlates. Much of this work makes a priori assumptions about how musical (or unmusical) stimuli are. Since our goal is to understand how low-level properties of auditory information give rise to the perception of auditory objects and, ultimately, the concept of musicality, the present study approaches the question from a less presumptive direction by initially asking subjects to identify sequences that are musical versus those that are not. Sequences were then analyzed to understand the features that guided behavioral ratings of musicality. In Exp. 1, subjects evaluated 50 ten-tone sequences according to how musical they thought they were. A unique corpus was designed that controlled for timbre, pitch content, pitch range, rhythm, note and sequence length, and loudness. Musicality ratings were on a scale of one (not musical) to five (very musical). Stimulus ratings showed significantly distinct groupings of musical versus non-musical sequences. To test the degree to which low-level organizational parameters affect musicality ratings, in Exp. 2 we manipulated the 7 most musical and 7 least musical sequences by changing auditory scene analysis (ASA) cues for a subset of tones. Changes in Amplitude and Timbre lead to a significant reduction in musicality ratings, whereas changes in the Attack of the tone onsets increased the musicality ratings. These results suggest that ASA cues have a direct influence over music processing. In an effort to explore whether similar neural structures are involved in processing music versus ASA cues, in Exp. 3 we used fMRI during a one-back memory task on the same stimuli from Exp. 2. We found that differences in processing musical versus nonmusical sequences correlated with a large area of auditory cortex and ACC. However, low-level ASA cue manipulations (vs. non-manipulated sequences) produced activation dorsolaterally; Timbre changes correlated with MFG and supramarginal gyrus, while Amplitude changes correlated primarily with SMA activation. These data provide evidence that distinct brain networks are involved in music processing as compared with auditory scene analysis. However, our behavioral data suggest that ASA cues can directly affect musicality judgments. Our future work will focus on resolving this apparent discrepancy between the behavioral and neuroimaging data.

Disclosures: A.S. Greenberg: None. **R. Randall:** None.

Nanosymposium

488. Auditory System: Circuits and Perception

Location: 143A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 488.06

Topic: D.02. Auditory

Title: The rhythm network: an exploratory neuroimaging analysis of beat perception and production

Authors: *D. E. ANDERSON[1], J. IVERSEN[2], D. CALLAN[3], A. D. PATEL[4], R.-A. MÜLLER[5];
[1]SDSU Brain Develop. Imaging Lab., Los Angeles, CA; [2]UCSD, San Diego, CA; [3]ATR Labs., Kyoto, Japan; [4]Tufts Univ., Boston, MA; [5]SDSU Brain Develop. Imaging Labratory, San Diego, CA

Abstract: The perception of beat, despite seeming easy and intuitive, is a complex cognitive and sensorimotor process, which underlies important mechanisms of temporal perception and motor control. Deficits in this ability domain have been shown to correlate with impairment in disorders such Parkinson's disease and developmental dyslexia. Neuroimaging studies suggest that beat perception (and moving to a beat) involve the interaction of auditory and motor regions, with a supportive network of prefrontal, parietal, and deep striatal areas. However, the interactions among these regions are not well understood. This study therefore aimed (i) to use activation (fMRI) and functional connectivity (fcMRI) techniques to confirm, and further define, the critical regions of this beat network and (ii) to employ task-based functional connectivity (fcMRI) methods to map the most important connections within this network, highlighting how they change dependent on task behavior and stimuli. We measured beat perception via a contrasting strong and weakly beat-inducing rhythms in a beat rating and a beat production/maintenance task. Expanding upon previous studies, we examined connectivity among a more complete set of brain regions then previously attempted (n=18). Activation results for 14 healthy, right-handed participants (4F, 24 ± 4.5yrs) were consistent with previous literature, and indicated that both beat perception and production recruit neural resources from a single network. Greater relative activation for strong beat was seen in bilateral auditory cortices, putamen, inferior parietal lobules, supplementary motor areas, as well as the anterior and posterior cingulate cortices. Preliminary functional connectivity analyses revealed a network whose connectivity was largely the same regardless of condition, with a few specific increases during the strong-beat condition of the production/maintenance task. Specifically, greater connectivity was found between the left prefrontal and both primary auditory and supplementary

motor areas, between the right premotor cortex and both the thalamus and the left auditory cortex, as well as between left and right anterior and posterior cerebellar lobes. Our findings depict a complex and dynamic beat perception and production network, whose function relies not simply on the increased recruitment of particular neural resources, but also in strategic changes in the communication between those resources and other critical network nodes.

Disclosures: D.E. Anderson: None. **J. Iversen:** None. **D. Callan:** None. **A.D. Patel:** None. **R. Müller:** None.

Nanosymposium

488. Auditory System: Circuits and Perception

Location: 143A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 488.07

Topic: D.02. Auditory

Support: NSERC RGPIN 217297-2010

Title: Direct current stimulation disrupts consolidation of auditory pitch discrimination learning

Authors: *R. MATSUSHITA[1,2], J. ANDOH[3], R. J. ZATORRE[1,2];
[1]Montreal Neurolog. Institute, McGill Univ., Montreal, QC, Canada; [2]Intl. laboratory for Brain, Music, and Sound Res. (BRAMS), Montreal, QC, Canada; [3]Dept. of Cognitive and Clin. Neurosci., Central Inst. of Mental Hlth. Mannheim, Mannheim, Germany

Abstract: Introduction: Transcranial direct current stimulation (tDCS) is known to modulate cortical activity in a polarity-specific manner; anodal tDCS increases cortical excitability and cathodal tDCS decreases it [1]. Several studies suggest tDCS induces an LTP-like effect [2] and in fact, involvement of tDCS in learning has been demonstrated at a behavioral level [3,4]. However, the role of tDCS on auditory learning is largely unknown. Here we address the effect of tDCS on a melody discrimination task. We targeted right auditory cortex because of its known role in tonal processing. We hypothesized that tDCS over this region would modulate learning over time. Methods: Twenty healthy adult participants were trained with a melody discrimination task over three consecutive days. We used micromelodies, which are melodies with pitch intervals that are smaller than one semitone. Learning to discriminate small pitch intervals with this task has been shown to involve changes in the right auditory cortex [5]. We implemented a psychophysical staircase procedure to establish a pitch discrimination threshold for performance. Baseline performance was measured on Day1. On Day2, participants received either anodal

tDCS or sham tDCS for 20 minutes. The active electrode was placed over right auditory cortex and the reference electrode on the contralateral supraorbital region. On Day3, participants did the same training task without tDCS. For the analysis, each individual's thresholds were converted into % change based on the performance on Day1. Results: Performance of the anodal tDCS group didn't significantly change over three days whereas the sham stimulation group showed significant learning by the end of the training, as expected [5]. In addition, no significant effect of anodal tDCS was observed during performance on Day2, suggesting that tDCS doesn't interfere with perception, but probably interferes with consolidation. Conclusion: We observed that anodal tDCS blocked learning consolidation overnight. This result is consistent with several studies showing that tDCS affects task performance offline, rather than online [3,6]. Our result supports this tDCS effect on between-session learning, and provides causal evidence for the importance of right auditory cortex to melody processing. Reference: 1. Nitsche M.A., Paulus W (2000) J Physiol, 527(3):633-639. 2. Nitsche M.A., et al (2003b). J Physiol, 553(1):293-301. 3. Nitsche M.A., et al (2003c). J Cog Neurosci, 15(4):619-626. 4. Reis J., et al (2009). Proc Natl Acad Sci, 106(5):1590-1595. 5. Zatorre R.J., et al (2012). Front Psychol, 3:1-17 6. Peters M.A., et al (2013). Neuropsychologia 51(7):1234-1239

Disclosures: **R. Matsushita:** None. **J. Andoh:** None. **R.J. Zatorre:** None.

Nanosymposium

488. Auditory System: Circuits and Perception

Location: 143A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 488.08

Topic: D.02. Auditory

Support: CIHR operating grant

Title: fMRI pattern analysis of played vs. perceived piano sequences in dorsal and ventral cortical streams

Authors: ***M. E. KLEIN**[1,4,5], A. HOLLINGER[2], R. J. ZATORRE[3,4,5];
[2]Ctr. for Interdisciplinary Res. in Music Media and Technol., [3]Psychology, [1]McGill Univ., Montreal, QC, Canada; [4]Cognitive Neurosci., Montreal Neurolog. Inst., Montreal, QC, Canada; [5]Intl. Lab. for Brain, Music, and Sound Res., Montreal, QC, Canada

Abstract: How does a musician know which action to execute to produce a desired sound? To examine this question, we used multivariate pattern analyses (MVPA), where algorithms learn to

decode input data and which has recently been used effectively with human functional magnetic resonance imaging (fMRI). In the field of auditory cognitive neuroscience, MVPA has been used to decode various perceptual and cognitive conditions including speaker identity, auditory imagery, and categorically-perceived musical intervals. However, less has been done to leverage the advantages of MVPA to examine auditory-motor interactions, which are a major component of both speech and musical processing. Here, we enrolled expert piano players to undergo fMRI scanning while (a) passively listening to various melodies, or (b) making matched fingering patterns on an MR-compatible piano keyboard. These musical actions were performed either with accompanying auditory feedback or in silence. The melodic triads, which were based on major or diminished chords, could be produced using combinations of the thumb, 3rd and 5th or thumb, 2nd and 4th digits. MVPA was performed on sound stimuli (ignoring motor condition) or motor condition (ignoring sound stimuli) to determine spatial dissociation/overlap between areas representing motor action or sound identity. These regions, hypothesized to be within the parietal and temporal lobes, respectively, are thought to contribute to the dorsal and ventral cortical streams, themselves comprising the "two streams" hypothesis of perception. We used a searchlight (roving sphere of voxels) methodology in order to localize pattern information to specific anatomical regions. Above-chance decoding was achieved by classifying either via sound or motion. Decoding based on fingering patterns highlighted a left hemispheric network, including regions of the parietal lobe, whereas decoding for sound showed a greater right hemisphere focus, including regions of the right superior temporal sulcus highlighted in earlier studies from our lab. Overall, classification based on sound or movement showed significant left/right and dorsal/ventral disparities, suggesting differential roles of these areas in an audio-motor integrative network.

Disclosures: **M.E. Klein:** None. **R.J. Zatorre:** None. **A. Hollinger:** None.

Nanosymposium

488. Auditory System: Circuits and Perception

Location: 143A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 488.09

Topic: D.02. Auditory

Support: EU Marie Curie Training Grant 'TRACKDEV'

Title: Musical training effects and cortical plasticity: Relationships with performance and training extent

Authors: D. CAREY[1], J. DIEDRICHSEN[2], A. LUTTI[3], M. SERENO[1], N. WEISKOPF[2], *F. DICK[1];
[1]Birkbeck/UCL Ctr. For NeuroImaging, London, United Kingdom; [2]UCL, London, United Kingdom; [3]Ctr. Hospitalier Universitaire Vaudois, Lausanne, Switzerland

Abstract: The human brain shows a remarkable capacity to adapt to experience. Long-term audio-motor experience such as musical training may spur structural change across areas including primary auditory cortex, primary motor cortex, cerebellum, and subcortical fibre tracts . However, few studies have compared musicians and non-musicians using quantitative metrics of brain structure, and assays of perceptual performance. We asked whether expertly trained violinists would show differences in cortical R1 (a quantitative proxy for myelination) within auditory and motor ROIs (auditory core and Heschl's gyrus; hand omega), as compared to an age-matched non-musician group. We assessed brain structure-behaviour relationships using a range of auditory psychophysical measures (thresholds for onset rise time, FM depth and AM depth) collected in the same participants. Using high resolution (0.512^3mm) multiparameter mapping techniques, we measured quantitative R1 (1/T1) rates at different cortical depths for each participant. We conducted both cortical-surface-based whole brain and ROI analyses, and found that violinists showed greater mean R1 at left (but not right) auditory core at mid cortical depths compared to non-musicians. Current analyses did not reveal evidence of group differences in mean R1 at hand area across either hemisphere. Thresholds for AM depth (but not FM depth or onset rise time) predicted mean R1 values within right auditory core and marginally at left core. We suggest our results support a model of cortical plasticity based on profiles of myelination that reflect expertise. Further, individual differences in behavioural sensitivity to fine temporal cues may serve to predict underlying differences in cortical structure.

Disclosures: D. Carey: None. **J. Diedrichsen:** None. **A. Lutti:** None. **M. Sereno:** None. **N. Weiskopf:** None. **F. Dick:** None.

Nanosymposium

488. Auditory System: Circuits and Perception

Location: 143A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 488.10

Topic: D.02. Auditory

Support: NIH Grant HD057522

Title: The language system is not required for processing musical structure

Authors: *E. FEDORENKO[1], J. H. MCDERMOTT[1], R. VARLEY[2];
[1]MIT, Cambridge, MA; [2]UCL, London, United Kingdom

Abstract: To interpret language or to appreciate music, we must understand how different elements - words in language, notes and chords in music - relate to one another. The processing of syntactic relations in these two domains has been argued to rely on partially overlapping pools of cognitive and neural resources (e.g., Maess et al., 2001; Koelsch et al., 2002; Levitin & Menon, 2003; Tillmann et al., 2003). But is this overlap functionally important? Several prior studies have asked this question by examining the processing of musical syntax in individuals with aphasia (e.g., Patel et al., 1998; Sammler et al., 2011) and have reported subtle deficits. However, these studies typically focused on individuals with relatively modest damage. We tested the processing of tonal structure in music in globally aphasic individuals with extensive perisylvian damage affecting both frontal and temporal / temporo-parietal components of the language network. These individuals have severe difficulties in linguistic processing, with an almost complete inability to produce / understand sentences, as assessed with sentence comprehension and grammaticality judgment tasks (e.g., Caramazza & Zurif, 1976; Linebarger et al., 1983). Three participants with global aphasia were asked to judge the well-formedness of a set of 180 melodies (90 melodies, with a "good" and a "sour" version each, distributed across two lists administered some time apart). The melodies were between 10 and 14 notes, with the "good" versions being tonal and ending in a tonic note with an authentic cadence in the implied harmony. The first five notes established a strong sense of key. In the sour versions, the pitch of one note was shifted up or down by 1 or 2 semitones subject to the constraints that 1) the note moved out of key and 2) the melodic contour did not change. Judgments were intended to reflect the detection of the key violation in the sour versions of the melodies. The task was pre-tested on 50 healthy control participants who on average responded correctly on 84.9% of trials (SD = 11.3; average d'=2.6), suggesting that the task was not too easy. The three patients had accuracies of 89.4, 90.1 and 94.4 (d' values: 3.13, 2.64, and 3.55), falling on the higher end of the performance range observed in controls. These results suggest that the processing of tonal structure in music remains intact even when the language system is severely impaired. These findings are consistent with recent neuroimaging studies that failed to observe responses to music stimuli in language-responsive cortex (Rogalsky et al., 2011; Fedorenko et al., 2012).

Disclosures: E. Fedorenko: None. **J.H. McDermott:** None. **R. Varley:** None.

Nanosymposium

488. Auditory System: Circuits and Perception

Location: 143A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 488.11

Topic: D.02. Auditory

Support: NDSEG Fellowship (32 CFR 168a) to E.A.P.

Title: The optimal time scale of statistical summary in human auditory perception

Authors: *E. A. PIAZZA[1], R. N. DENISON[2], T. SWEENY[3], J. SHEYNIN[1], M. A. SILVER[1], D. WHITNEY[1];
[1]Univ. of California, Berkeley, Berkeley, CA; [2]New York Univ., New York, NY; [3]Univ. of Denver, Denver, CO

Abstract: Humans frequently encounter complex groups of sensory stimuli (e.g., people in a crowd, cars in traffic, notes in an aria) and are able to quickly perceive them. To rapidly combine numerous visual features into a coherent percept, humans generate representations based on summary statistics (e.g., average size, color, facial expression, etc.), which provide sufficient information to perceive the gist of the group. Previously, we found that summary statistics are also important for auditory perception, in particular for representing average pitch information from tone sequences over time. Listeners could accurately estimate the average pitch of a temporal sequence of pure tones, despite very limited ability to recall information about specific individual member tones (discriminating them from non-heard tones and determining their positions in the sequence). These results indicated that summary statistical coding is important for condensing complex information not only from visual scenes but from auditory ones as well, and likely reflects a more generalized representational strategy in the brain. Here, we investigated whether auditory summary representation is temporally tuned: does it operate most effectively at a particular time scale? The answer to this question will shed light on whether auditory statistical summary might be optimized for a particular perceptual function (e.g., compressing pitch information in sentences, music comprehension, etc.). In a psychophysical experiment, we presented tone sequences that varied either in tone duration (with a constant interstimulus interval of 0 ms) or in the interstimulus interval (ISI) between consecutive tones (with a constant tone duration of 50 ms). In each trial, subjects heard a sequence and were asked to report whether a subsequent probe tone was higher or lower than the average pitch of the sequence. We found that both tone duration and ISI influence subjects' ability to estimate the average pitch of a tone sequence, with performance approaching a peak at values corresponding to an overall stimulus presentation rate of approximately 5-6 Hz. Interestingly, this rate is consistent with the average syllabic rate of natural human speech, raising the intriguing possibility that summary statistical perception may be particularly useful for estimating emotional tone, gender, or other features from the average pitch of sequences of syllables.

Disclosures: E.A. Piazza: None. **R.N. Denison:** None. **T. Sweeny:** None. **J. Sheynin:** None. **M.A. Silver:** None. **D. Whitney:** None.

Nanosymposium

488. Auditory System: Circuits and Perception

Location: 143A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 488.12

Topic: D.02. Auditory

Support: NIH Grant DC004263

Title: Multiple neural representations of ambiguous auditory input are simultaneously maintained even when only one appears in perception

Authors: *E. S. SUSSMAN[1], A. S. BREGMAN[2], W. LEE[3];
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[3]Albert Einstein Col. of Med., Bronx, NY

Abstract: If you were walking on a busy city street, you might hear people talking as they walk past you, cars driving by, and bursts of a jackhammer at the construction site. In the presence of all the competing sound sources, the ability to perceive these events as discrete sound streams relies on multiple neural mechanisms that organize the mixture of sound input entering the ears. Most studies have focused on mechanisms that contribute to either integrating the sounds that belong together into one perceptual stream (integration) or segregating the sounds to different sources (segregation). However, little is known about mechanisms that allow us to perceive individual sound sources within a dynamically changing auditory scene, when the input may be ambiguous, and heard as either integrated or segregated. This study focused on the question of how neural representations of auditory input accommodate to the changing multi-source acoustic environment. We presented listeners with ambiguous input and cued them to switch between tasks that used the integrated and segregated percepts. Electrophysiological measures indicated which organization was currently maintained in memory. If mutual exclusivity at the neural level was the rule, attention to one of two possible organizations would preclude neural representation of the other. However, results indicated that both organizations (integrated and segregated) were simultaneously maintained in memory regardless of which task was performed. Thus, focusing on one task did not suppress representation of the alternative, unattended organization. In noisy environments, such as walking on a city street, rapid and flexible adaptive processes are needed to help facilitate rapid switching to different sound sources in the environment. Having multiple representations available to the attentive system would allow for such flexibility. Rather than a competitive model of the system, in which the multiple organizations are in competition for a winner-take-all resolution, our results support an alternative theory in which multiple

representations are simultaneously maintained, allowing rapid scanning of various events in a busy environment.

Disclosures: **E.S. Sussman:** None. **A.S. Bregman:** None. **W. Lee:** None.

Nanosymposium

489. Striate Cortex

Location: 206

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 489.01

Topic: D.04. Vision

Support: NIH Grant EY012114

Title: Correlation of membrane potential between neurons in visual cortex of behaving mice

Authors: S. ARROYO[1], C. BENNETT[2], *S. HESTRIN[1];
[1]Dept of Comparative Med., Stanford Sch. of Med., STANFORD, CA; [2]Stanford Sch. of Med., Stanford, CA

Abstract: Correlation of neuronal spiking is thought to be essential for coding and for processing information in the visual cortex. However, the correlation of the underlying membrane potential of visual cortical cells has not been studied in awake animals. To address this issue, we obtained whole-cell recordings from pairs of L2/3 pyramidal neurons in V1 of unanaesthetized mice. We found that the membrane potentials of neighboring cells are remarkably correlated during quiet wakefulness (r = 0.7). This high degree of correlation is mostly driven by large (10-20 mV), slow (2-5 Hz) fluctuations of membrane potential that are also reflected in the LFP. Using voltage-clamp recordings, we show that these slow fluctuations are generated by an increase in excitation followed by an increase in inhibition (lag ~10 ms). During locomotion, these fluctuations are suppressed, and the membrane potential correlation is reduced (r = 0.4). To determine the space constant of the correlated activity observed during quiet wakefulness, we determined the correlation between simultaneous LFP recordings at a range of distances (both in microns and in visual field location). As we increased the distance between the two LFP recordings, we found that correlations decreased with a space constant of about 50° in visual space. These data imply that the membrane potential of L2/3 neurons is highly correlated over a wide swath of the visual field. During visual stimulation, correlations were shifted from a low-frequency (1-10 Hz) band to higher frequencies (15-40 Hz). However, despite high Vm correlations on a relatively long timescale, we found that the synaptic inputs

generating action potentials were predominantly private, consistent with low measurements of spike synchrony in sensory cortex of un-anesthetized animals.

Disclosures: **S. Arroyo:** None. **S. Hestrin:** None. **C. Bennett:** None.

Nanosymposium

489. Striate Cortex

Location: 206

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 489.02

Topic: D.04. Vision

Support: Wellcome Trust Grant 095667

Wellcome Trust Grant 095668

Title: The nature of cortical variability

Authors: ***I.-C. LIN**, M. OKUN, M. CARANDINI, K. D. HARRIS;
Univ. Col. London, London, United Kingdom

Abstract: The responses of sensory cortex to repeated presentations of a stimulus are highly variable, and this variability is correlated between neurons. Variability has long been assumed to be additive, but recent work (Goris et al Nature Neurosci 2014; Ecker et al, Neuron 2014) suggests that it may involve a multiplicative gain change. We asked whether the nature of variability is additive or multiplicative, and whether it can be summarized by a few factors affecting an entire neuronal population. We analyzed the trial-by-trial activity of large populations in primary visual cortex (V1) of anesthetized cats in response to contrast-reversing gratings and plaid stimuli (Busse et al, Neuron 2009). To describe the data, we used a simple "affine" model that comprises two sources of variability: a multiplicative component that invests all neurons in proportion to their instantaneous sensory drive (i.e., a variable response gain), and an additive component that is independent of the sensory drive but affects individual neurons to different degrees. This simple affine model captured the structure of trial-to-trial variability in the entire population. It predicted how on individual trials the population responses to gratings and plaids are both shifted additively and scaled multiplicatively. Furthermore, the affine model predicted the complex relationship between correlation and individual neuronal tuning, providing a simple and intuitive explanation for well-known phenomena observed at the level of neuronal pairs, such as the stimulus-dependence of noise correlations. Cross-validation analysis showed

that the affine model performed better than either multiplicative or additive models alone.The sizes of additive and multiplicative fluctuations varied both between experiments and from moment to moment within an experiment, indicating that the relative importance of the two fluctuations is state-dependent. We conclude that the nature of cortical variability is best understood at the population level, where it can be captured by a simple mathematical model. This model reveals that cortical variability is dominated by both multiplicative and additive fluctuations. In behaving animals, 'top-down' behavioral factors such as attention control the gain of stimulus responses in sensory cortex. We suggest that this top-down modulation may rely on the same circuitry that is responsible for the trial-to-trial fluctuations in gain that we observed here.

Disclosures: I. Lin: None. **M. Okun:** None. **M. Carandini:** None. **K.D. Harris:** None.

Nanosymposium

489. Striate Cortex

Location: 206

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 489.03

Topic: D.04. Vision

Support: Wellcome Trust

EPSRC

ERC

MRC

Title: Choristers and soloists in a cortical population

Authors: *M. OKUN[1], L. COSSELL[1], M. F. IACARUSO[1], H. KO[1], P. BARTHO[2], S. B. HOFER[1], T. D. MRSIC-FLOGEL[1], M. CARANDINI[1], K. D. HARRIS[1];
[1]UCL, London, United Kingdom; [2]Inst. of Exptl. Medicine, Hungarian Acad. of Sci., Budapest, Hungary

Abstract: Characterizing the firing patterns produced by large neuronal populations - and their relationship to underlying circuitry - is an essential step toward understanding information processing in the cortex. Local cortical populations exhibit coordinated modulations in firing rate, leading to the view that individual neurons act in concert as "obedient members of a huge

orchestra". According to this view, neurons in sensory cortex are arranged in ensembles determined by sensory selectivity, with each neuron displaying little autonomy relative to the corresponding ensemble. To characterize the relationship of individual neurons to population activity, we analyzed population recordings from rodent sensory cortex obtained from multisite silicon probes. We also analyzed neuronal populations that were first recorded in vivo using 2-photon imaging with OGB-1 AM dye, followed by later assessment of pairwise connectivity by multiple intracellular recordings in vitro. We found that the degree to which a neuron couples to population activity ranges along a continuum from neurons whose firing is strongly tied to mean population rate ("choristers"), to others that fire independently of it ("soloists"). Population coupling differed both between and within cortical cell classes, and was conserved between spontaneous activity and sensory stimulation, indicating that it represents an invariant property of each neuron, rather than its response to a specific set of stimuli. Soloists differed from choristers in a number of ways. First, their response to visual stimuli involved little increase in firing rate. Second, they were driven less effectively by optogenetic activation of layer 5 pyramidal cells, indicating lower functional connectivity. Third, they were less likely to receive synapses from neighboring neurons, indicating decreased synaptic connectivity. To characterize how well knowledge of these factors can summarize the structure of population activity, we constructed a simple model based only on (1) each neuron's mean firing rate; (2) each neuron's population coupling; and (3) the distribution of population rate. This model - parametrized by 3N numbers - sufficed to predict much of the order N^2 pairwise correlations observed in the population. We conclude that a surprisingly simple, single dimension characterizing each neuron's invariant relationship to a larger population, is central to explaining seemingly complex population patterns in terms of underlying circuit variables.

Disclosures: **M. Okun:** None. **L. Cossell:** None. **M.F. Iacaruso:** None. **H. Ko:** None. **P. Bartho:** None. **S.B. Hofer:** None. **T.D. Mrsic-Flogel:** None. **M. Carandini:** None. **K.D. Harris:** None.

Nanosymposium

489. Striate Cortex

Location: 206

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 489.04

Topic: D.04. Vision

Support: DFG Exec 307

ERC Grant 281886 PERCEPT

Title: Effects of locomotion on pre-cortical and cortical neural populations in the mouse visual system

Authors: *S. ERISKEN, A. VAICELIUNAITE, O. JURJUT, M. FIORINI, S. KATZNER, L. BUSSE;
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Abstract: Neural responses in primary visual cortex (V1) depend not only on sensory input but are also profoundly modulated by behavioral context. One such behavior is locomotion, shown in mice to enhance the gain and reduce the variability of cortical neurons. Beyond global state-dependent changes, V1 single neurons integrate locomotory and visual signals in complex ways and can exhibit tuning for running speed. Response modulation by locomotion along the visual pathway is currently thought to be restricted to cortical neurons, however, little is known about how locomotion affects cortical populations and pre-cortical processing stages. Using extracellular recordings from multiple neurons in head-fixed mice running on a spherical treadmill, we investigated the influence of locomotion on neural populations in area V1 and in the dorsal lateral geniculate nucleus (dLGN). We measured pupil position and size using camera-based eye tracking under infrared illumination. We assessed how locomotion shapes population responses in V1 upper layers by computing pairwise spike count correlations between simultaneously recorded neurons. During spontaneous activity, locomotion de-correlated population activity, reducing both variability of individual cells and shared variability between pairs. Interneuronal correlations, during both locomotion and stationary periods, varied as a function of orientation and run-speed tuning similarity, with highest correlations for pairs preferring similar orientations and running speeds. Remarkably, despite variable speed preferences within the population, locomotion globally reduced interneuronal correlations across all degrees of run-speed tuning similarity and even more so for similarly tuned pairs. Given such prominent effects of locomotion on cortical populations, we next re-assessed if these effects are indeed restricted to cortex. Contrary to current understanding, we discovered that locomotion modulated responses as early as the dLGN. Individual cells enhanced their activity around locomotion onset and were tuned for run-speed. While locomotion modulated dLGN response magnitude, locomotion, unlike in cortex, did not de-correlate population activity . Finally, we found that pupil size closely followed running speed. While changes in pupil size could not account for locomotion-based response enhancements, they instead seemed to serve as a reliable marker for behavioral state. These findings document previously unknown and far-reaching effects of locomotion throughout the early visual system and demonstrate that state-dependent changes can exert powerful influences beyond response gain modulations.

Disclosures: **S. Erisken:** None. **A. Vaiceliunaite:** None. **O. Jurjut:** None. **M. Fiorini:** None. **S. Katzner:** None. **L. Busse:** None.

Nanosymposium

489. Striate Cortex

Location: 206

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 489.05

Topic: D.04. Vision

Support: NIH Grant EY019049

NIH Grant EY022478

Kirchgessner Foundation

Title: Cortical inhibition determines the orientation selectivity in different cell types

Authors: *Y.-T. LI[1], B.-H. LIU[2], L. I. ZHANG[2], H. W. TAO[2];
[1]Physiol. and Biophysics, [2]USC, Los Angeles, CA

Abstract: In primary visual cortex (V1), there are mainly two types of neurons in terms of receptive field functions, simple cells and complex cells. There has been some evidence showing that simple cells are more orientation selective than complex cells. However, whether and how this difference in the level of orientation selectivity is attributed to differential interplay between synaptic excitation and inhibition is unknown. In the current study, we combined in vivo loose-patch recording, whole-cell current-clamp and voltage-clamp recordings to address this question in layer 2/3 of mouse V1. Indeed, our loose-patch and current-clamp recordings revealed that simple cells were more orientation selective than complex cells at levels of both spike response and membrane potential response. Using whole-cell voltage-clamp recordings, we demonstrated that simple and complex cells differed in the level of orientation tuning of synaptic inhibition, while their synaptic excitation had similar tuning selectivity. Specifically, inhibition was more broadly tuned than excitation in simple cells, but more narrowly tuned than excitation in complex cells. Therefore our data suggest that it is the cortical inhibition rather than excitation that determines differential orientation selectivity in different cell types.

Disclosures: **Y. Li:** None. **B. Liu:** None. **L.I. Zhang:** None. **H.W. Tao:** None.

Nanosymposium

489. Striate Cortex

Location: 206

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 489.06

Topic: D.04. Vision

Title: An optogenetic and double whole-cell recording analysis of functional connections from GABAergic to pyramidal neurons in layer 2/3 of the mouse visual cortex, *in vivo*

Authors: *M.-S. SAFARI, R. KIMURA, T. TSUMOTO;
Lab. For Cortical Circuit Plasticity, RIKEN Brain Sci. Inst., Wako/Saitama, Japan

Abstract: GABAergic inhibition plays a crucial role in shaping responses of cortical pyramidal neurons to visual stimuli, but it is not clear whether a single GABAergic neuron or a certain number of GABAergic neurons as a group play such a role and if the latter is the case how many. In this study we addressed these questions by using the in vivo two-photon targeted paired whole-cell recording method combined with optogenetic activation of a group of GABAergic neurons. Inhibitory postsynaptic currents (IPSCs) were recorded from excitatory neurons following action potentials of a single GABAergic neuron or optogenetic activation of all GABAergic neurons in a certain area of the cortex. Results showed that IPSCs induced by each action potential of single GABAergic neurons were in the range of 4.5±0.4 pA (n=4). Optogenetic activation of all GABAergic neurons in the cortical area of around 200 μm in diameter increased IPSCs to 98±8.3 pA (n=9). We estimated that at least 22 GABAergic neurons functionally connect to a single pyramidal neuron in layers 2/3 of the visual cortex. We also found that activation of single GABAergic neurons is not enough for changing orientation tuning of visual responses of pyramidal neurons, but optogenetic stimulation of a group of surrounding GABAergic neurons can change orientation tuning.

Disclosures: **M. Safari:** None. **R. Kimura:** None. **T. Tsumoto:** None.

Nanosymposium

489. Striate Cortex

Location: 206

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 489.07

Topic: D.04. Vision

Support: NIH Grant EY022853-02

Title: Optogenetic stimulation of monkey primary visual cortex

Authors: *M. M. CHERNOV, G. CHEN, A. W. ROE;
Psychology, Vanderbilt Univ., Nashville, TN

Abstract: Despite its uniform appearance to the naked eye, primate visual cortex is organized into functional domains that are responsible for extracting visual features such as orientation, color and position of objects in space. These domains have been observed using both electrophysiological and imaging methods, and must ultimately be coded within the underlying neuronal cytoarchitecture. Such local connections between ocular dominance (OD) columns and orientation domains have indeed been found in layers 2/3 of primate primary visual (VI) cortex (Malach et al., Proc. Natl. Acad. USA, 1993). We used a combination of optogenetic stimulation (OS) and intrinsic optical signal imaging (IOSI) to determine whether these connections can be revealed in vivo using focal stimulation of specific functional domains, and to explore the interaction between such activation and the state of the network, which we altered by presenting various visual stimuli. Experiments were carried out in two anesthetized and paralyzed macaques, previously injected in V1 with a channelrhodopsin-carrying lentiviral vector. Visual stimulation was accomplished by presenting vertical and oblique full-screen square gratings with the goal of activating ocular dominance stripes and orientation domains. We found that the spatial patterns of neural activation revealed by IOSI following OS alone were determined in part by the functional connections of the area expressing ChR2. Thus, stimulation of the OD column containing ChR2 led to activation of neighboring same-eye OD columns. The opposite-eye OD columns, on the other hand, were not activated. When optogenetic stimulation was combined with visual stimulation, the observed neural activation patterns revealed a complex interaction between the two stimuli, demonstrating facilitation or inhibition depending on the type of visual stimulus presented and the timing of visual and optogenetic stimulus presentation. These results are intriguing and contrast with a linear summation of the two types of stimulation reported in tree shrews (Huang et al., J. Neurosci., 2014). In conclusion, (1) we demonstrate the use of optogenetics to stimulate specific functional domains in area V1 of a non-human primate. (2) We show that changes in neural activation as revealed by IOSI following optogenetic stimulation are determined in part by the underlying neural architecture (in this case, the OD columns) and in part by the state of the network which, when modified by presentation of a relevant visual stimulus may facilitate or oppose the influence of the optogenetic stimulus.

Disclosures: **M.M. Chernov:** None. **G. Chen:** None. **A.W. Roe:** None.

Nanosymposium

489. Striate Cortex

Location: 206

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 489.08

Topic: D.04. Vision

Support: NIH Grant EY021827

NIH Grant EY020673

Gatsby Charitable Foundation

Title: Normalization through local excitation and inhibition in macaque primary visual cortex

Authors: *J. J. NASSI[1], M. C. AVERY[1], A. H. CETIN[1], A. W. ROE[2], J. H. REYNOLDS[1]; [1]Salk Inst. For Biol. Studies, La Jolla, CA; [2]Vanderbilt Univ., Nashville, TN

Abstract: Normalization has been proposed as a canonical cortical computation operating across a wide range of sensory modalities, brain areas and species. Its defining property is that the excitability of a neuron is inversely proportional to the overall activity level of the network. In primary visual cortex (V1) this can account for a wide range of non-linear response properties, including the sigmoidal shape of the contrast response function and cross-orientation suppression. One of the key components of normalization is a broadly-tuned, divisive input signal that scales with stimulus contrast. The underlying circuitry of this so-called "normalization pool" remains poorly understood. Here, we measured the causal effects of locally-generated excitation and inhibition on spontaneous and visually-evoked responses in V1 of alert, fixating macaque monkeys. Optogenetic depolarization of excitatory neurons produced both facilitation and suppression of spontaneous activity, consistent with the interpretation that optogenetic stimulation activated the normalization circuit, causing both increased excitatory drive as well as indirect divisive inhibition. Increased recruitment of both excitation and inhibition with stimulation intensity produced non-linear response properties similar to those typically observed with increased luminance contrast. Accordingly, we hypothesized that simultaneous visual and optogenetic stimulation should interact as predicted by a normalization model. According to the model, optogenetic stimulation added excitatory drive and divisive suppression to the visual contrast response function. The ratio of optogenetic induced excitation and inhibition was assumed to vary across the population and scaled with both stimulation intensity and contrast. As predicted by the model, simultaneous visual and optogenetic stimulation produced sub-additive responses that were well characterized by a weighted sum of the individual responses. The weights depended strongly on the relative intensity of visual and optogenetic stimulation, ranging from near-equal averaging to winner-take-all. We observed a range of effects of optogenetic stimulation on the visual contrast response functions, all of which

were well accounted for by the model. These results suggest that optogenetic depolarization of excitatory neurons activates the neural elements that mediate normalization in the primate cortex.

Disclosures: **J.J. Nassi:** None. **M.C. Avery:** None. **A.H. Cetin:** None. **A.W. Roe:** None. **J.H. Reynolds:** None.

Nanosymposium

489. Striate Cortex

Location: 206

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 489.09

Topic: D.04. Vision

Support: NIMH Intramural

Whitehall Foundation

NSF GRFP DGE-0909667

Title: Sensory stimulation and attentional allocation evoke opposing patterns of columnar activation in primary visual cortex

Authors: ***M. A. COX**[1], D. A. LEOPOLD[2], A. V. MAIER[1];
[1]Dept. of Psychology, Vanderbilt Univ., Nashville, TN; [2]NIMH, Bethesda, MD

Abstract: Anatomical studies suggest that the laminar pattern of primary visual cortex (V1) reflects distinct functional organization. Retinogeniculate neurons carrying sensory information synapse predominantly in granular layer 4. Descending connections, which relay modulatory signals related to cognitive processes such as directed attention, memory, and planned action, specifically avoid this middle layer. However, direct neurophysiological evidence for this spatial segregation between sensory and modulatory signals remains scarce. Here, we compare V1's laminar pattern of neural activation evoked by bottom-up sensory stimulation to the laminar pattern following top-down attentional allocation. We recorded spiking activity and current source density (CSD) in two macaque monkeys across all cortical layers using a linear microelectrode array. Monkeys were required to fixate while grating stimuli were presented inside the receptive field of the recorded neural population. Confirming earlier studies, we found that stimulus onset evoked widespread spiking activity starting in layer 4, which was marked by a current sink of similar latency. We contrasted this basic sensory response pattern with the laminar profile of activity following the presentation of an endogenous attention cue several

degrees away from the borders of the receptive field. Consistent with previous work, we observed a consistent increase in spiking responses across all layers when the animals' attention was directed towards the stimulus. However, preceding this attention-related response enhancement and immediately following the cue onset, we observed a profound transient decrease in neuronal activity. This brief drop in spiking activity was observed across the entire cortical column. CSD analysis revealed two current sinks above and below layer 4, resembling the inverse of the current pattern observed immediately following visual stimulation. These observations, taken together, suggest that attentional cuing leads to a transient decrease of activity in stimulated sensory cortex that might be mediated by feedback projections to the extragranular layers.

Disclosures: **M.A. Cox:** None. **D.A. Leopold:** None. **A.V. Maier:** None.

Nanosymposium

489. Striate Cortex

Location: 206

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 489.10

Topic: D.04. Vision

Support: CIHR grant (#53346)

CIHR Grant (#108-18)

CIHR Grant (#125686)

Title: Real-time modulation of ocular dominance adults

Authors: *R. F. HESS, A. REYNAUD, J. ZHOU;
McGill, Montreal, QC, Canada

Abstract: Using a dichoptic spatial phase combination paradigm that assessing the relative contribution that each eye makes to the binocular percept (ocular dominance), we have shown previously that 2.5 hours of patching can result in a short-term enhancement of the patched eye's dominance. Similar effects can be obtained with translucent occlusion, so it is not due to an interocular change in mean luminance. Here we ask what the spatial determinants are for modulating the ocular dominance in humans using short-term monocular deprivation. Observers dichoptically viewed movies that last for 2.5 hours in which the spatial information in one eye's view had been altered. We measured each eye's contribution to the binocular percept before and

after 2.5 hours of movie viewing using the dichoptic spatial phase task. Scrambling the spatial phases in one eye's view had no effect on ocular dominance, suggesting features constructed from phase-aligned components are unimportant in this regard. At the level at which these changes in dominance occurs, only the Fourier amplitude spectrum is important. To verify this we show that graded changes to the magnitude of the amplitude spectrum result in graded changes in ocular dominance. To ascertain whether different parts of the amplitude spectrum are more important than others, we compared highpass with lowpass filtering and show that only the latter affects dominance. Short-term changes in ocular dominance in adults can be obtained by altering the contrast of high spatial frequency components seen by one eye.

Disclosures: R.F. Hess: None. **A. Reynaud:** None. **J. Zhou:** None.

Nanosymposium

489. Striate Cortex

Location: 206

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 489.11

Topic: D.04. Vision

Title: Short-term monocular deprivation disrupts interocular balance in adult macaque visual cortex

Authors: *D. Y. TS'O[1], M. BEGUM[1], B. T. BACKUS[2];
[1]Dept of Neurosurg., SUNY - Upstate Med. Univ., SYRACUSE, NY; [2]Grad. Ctr. for Vision Res., SUNY Col. of Optometry, New York,, NY

Abstract: _Short-term monocular deprivation (patching one eye for 2.5 hours) markedly alters interocular balance in adult humans, as measured using psychophysical procedures (Lunghi et al 2011; Zhou et al 2013). In behavioral tests of binocular integration, the relative contribution from the previously patched eye (PPE) was elevated for more than an hour after the patch was removed. _We have now observed via functional imaging a corresponding alteration of interocular balance in anesthetized macaque primary visual cortex (V1). Throughout this entire experiment, neural activity in V1 was measured with intrinsic signal optical imaging while a visual stimulus was presented. This stimulus, a dynamic grating changing in orientation and direction of motion second by second, was selectively gated to one eye or the other via shutters. After measuring baseline ocular dominance column (ODC) maps, one eye was patched while the other eye continued to view the same stimulus and functional imaging continued. This monocular deprivation phase lasted for 3 hours, whereupon the patch was removed. Visual

stimulation and ODC imaging continued for another 2 hours. _The imaging data were analyzed for ODC maps and for ODC signal strength (expressed as fractional reflectance change), as well as for response maps and signal strengths for each individual eye. A correlation coefficient was computed between the baseline ODC map and signal, and subsequently measured maps and signals. The baseline data showed a strong ODC map and contributions from each eye. During the 3 hours of patching, both the ODC map and the unpatched eye's effect steadily decreased, to about 40% of baseline. When the patch was removed, the ODC map returned to near-baseline within several minutes. Remarkably, immediately upon unpatching, the unpatched eye response was driven down further to only ~25% of baseline, while the PPE response was more than 2X the unpatched eye response. Over the next hour unpatched eye response, PPE response and overall ODC pattern all returned to near-baseline levels. _The weak response for the unpatched eye during and immediately after patching was striking. It cannot be explained by adaptation in the eye or cortex given that responses returned to baseline after unpatching. This preliminary result matches the previous psychophysical studies and suggests a dynamic mechanism for regulating interocular balance and gain, one that is likely cortical in origin.

Disclosures: D.Y. Ts'o: None. **M. Begum:** None. **B.T. Backus:** None.

Nanosymposium

489. Striate Cortex

Location: 206

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 489.12

Topic: D.04. Vision

Support: Wellcome Trust Studentship WT100931AIA

NIH Intramural Program

Title: Temporal dynamics of disparity extraction consistent with a single computation underlying stereopsis

Authors: S. HENRIKSEN[1], *B. G. CUMMING[2], J. C. A. READ[1];
[1]Inst. of Neurosci., Newcastle Univ., Newcastle, United Kingdom; [2]Natl. Eye Inst., Natl. Eye Institute, NIH, Bethesda, MD

Abstract: A long-standing problem in visual neuroscience is the stereo correspondence problem - how does the brain match features from the left eye with that of the right? Recently, Doi et al.

(2011, 2013) suggested that two distinct processes are involved in stereoscopic vision and in the resolution of the correspondence problem: a matching computation and a correlation computation. They suggest that these computations preferentially activate sustained and transient channels, respectively. The evidence for this comes from an intricate and ingenious stimulus called the half-matched random dot stereogram, where half the dots have the same contrast in each eye (i.e. are binocularly matched) and half have opposite contrast in each eye (i.e. are binocularly mismatched). Doi et al. showed that psychophysical performance changes in response to dynamic half-matched stereograms as one increases the presentation rate. They argued that this reflects a shift from a match-based computation at low presentation rates (corresponding to sustained channels) to a correlation-based computation at high presentation rates (corresponding to transient channels). We explore this interpretation with a new experiment, in which each video frame of the dynamic random dot stereogram consists entirely of either matched or mismatched dots. The dots were always updated at a high frequency (120 Hz), which should eliminate the contribution of the slow matching process. We varied the frequency at which matched and unmatched frames were alternated. We show that human observers are able to detect depth in these stimuli for correlation alternation rates up to around 15 Hz. This suggests that the correlation mechanism can detect disparity even in these stereograms. We propose that these findings can be accounted for by the temporal integration within the correlation mechanism, and that this parsimonious interpretation can be extended to the findings of Doi et al. Thus, a single mechanism is consistent with the evidence reported here and with that of previous experiments.

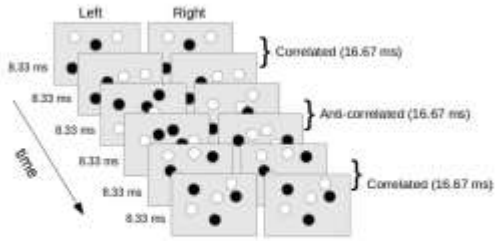

Figure 1: Illustration of stimulus employed. The dots were always updated at 120 Hz, while the rate at which correlation reversed was manipulated (from 3.75 Hz up to 60 Hz).

Disclosures: **S. Henriksen:** None. **B.G. Cumming:** None. **J.C.A. Read:** None.

Nanosymposium

489. Striate Cortex

Location: 206

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 489.13

Topic: D.04. Vision

Support: NEI EY022116 (JW)

NINDS NS078396 (JP)

Title: Using conscious visual perception to quantify the effect of electrical stimulation of human cerebral cortex

Authors: *J. WINAWER[1], J. PARVIZI[2,3];
[1]Psychology, New York Univ., New York, NY; [2]Neurol. and Neurolog. Sci., [3]Stanford Human Intracranial Cognitive Electrophysiology Program (SHICEP), Stanford Univ., Stanford, CA

Abstract: **Purpose** Electrical stimulation of the human brain has been used for a century as a means to explore the brain correlates of thought and behavior. Colorful perceptual and behavioral observations have been reported with focal electrical stimulation. More recently, electrical stimulation of the human brain has also been used as a means to alter the pathological functions of specific circuits, thereby alleviating the clinical signs and symptoms of neurological and psychiatric disorders such as Parkinson's disease and depression. Understanding the extent of human cortical tissue recruited by electrical stimulation with conventional settings is critical for interpretation of scientific results and clinical measurements. **Methods** To explore the spatial scale over which electrical charge delivery recruits cortical tissue, electrical stimulation was delivered to visual cortex of patients in a clinical setting with implanted subdural electrodes. Sufficient stimulation of occipital cortex elicits a spatially localized visual sensation, called a "phosphene". During stimulation, subjects had their eyes open and fixated the center of a polar grid displayed on a computer screen. Immediately after stimulation, patients were asked to draw their phosphene using a track-pad and to provide a numerical rating corresponding to the subjective intensity of the phosphene. The amount of charge delivered was varied by modulating the pulse width, frequency, and amplitude of stimulation. Phosphenes drawn by the patients were mapped on to surface representations on the cortex. The mapping was created by identifying nodes on the cortical surface whose population receptive field centers, measured with functional magnetic resonance imaging (Dumoulin and Wandell, 2008), were inside the contour of the phosphene. The combined surface area of all such nodes for a given phosphene was taken as the area of responsive cortex. **Results and Conclusion** The size of the phosphene varied depending on the amount of charge and the location of the electrode. For a given stimulation site, greater charge delivery led to larger phosphenes, as well as higher ratings of subjective intensity. Across sites, phosphenes were larger when the stimulated electrode was on the surface of peripheral V1 compared to foveal V1. Importantly, however, when the phosphenes were projected onto the cortical surface, the area of activation was approximately equal for peripheral and foveal stimulation for a given level of charge. The results indicate that the surface area of responsive cortex, unlike the size of the phosphene, is approximately invariant to the location of stimulation.

Disclosures: **J. Winawer:** None. **J. Parvizi:** None.

Nanosymposium

490. Psychosocial Stress and the Brain

Location: 140A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 490.01

Topic: E.05. Stress and the Brain

Support: NARSAD Grant #17830

NIH Grant 1P20GM103691

AHA Grant 13BGIA14370026

Title: Critical role of the locus coeruleus-norepinephrine system in social stress-induced hypertension

Authors: ***C. M. LOMBARD**, C. S. WOOD, L. WILSON, S. K. WOOD;
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Abstract: Psychosocial stress precipitates psychiatric disorders (eg, depression) as well as cardiovascular disease. The coping response plays a role in susceptibility to stress, however the underlying mechanisms are unknown. We previously identified two distinct phenotypic responses to social stress in rats, characterized by either passive coping (eg, supine postures) or active coping behaviors (eg, upright postures, counterattacks). The passive phenotype was associated with endocrine and behavioral characteristics resembling depression and decreased heart rate variability indicating greater resting sympathetic tone, while the active phenotype remained resilient to these changes. The locus coeruleus (LC) plays a critical role in the acute stress response and long-term adaptation to stress. It is the major source of norepinephrine in the brain, and LC dysregulation is implicated in depressive disorders. Furthermore, LC projections to the RVLM and DMV may also mediate the cardiac sympathetic stress response. Importantly, the sympathetic system exerts an excitatory influence on inflammatory cytokines linked to depressive disorders and cardiovascular disease, providing a mechanism by which stress-induced changes in LC activity may contribute to this comorbid condition. The present study tested the hypothesis that a partial lesion of the LC using the selective noradrenergic neurotoxin DSP-4 impacts the behavioral and cardiovascular consequences of social stress. Male Sprague Dawley rats were treated with DSP-4 (400 ug/rat, ICV) or vehicle 1-wk prior to 7 exposures (30 min/day)

to social stress or control. 24-hr ECG/pressure telemetry revealed that vehicle-treated passive rats showed persistent elevations in resting systolic pressure after only 2 defeat exposures compared with control and active coping rats. Furthermore, passive rats also exhibited increased anhedonic behaviors compared with controls as measured by a sucrose preference test. DSP-4 blocked the elevated pressure in passive rats, but had no effect on stress-induced anhedonia. In a separate subset of rats we identified exaggerated stress-induced IL-6 release in passive rats compared with control and active rats and ongoing studies are evaluating the impact of DSP-4 on this immune response. Active coping rats, on the other hand, did not show persistent pressor or anhedonic responses. Taken together, these data suggest that the LC may play a critical role in stress-induced increases in systolic pressure in susceptible passive rats. Therefore, exaggerated LC activity as a result of social stress exposure may predispose individuals to depressive-cardiovascular disease comorbidity.

Disclosures: C.M. Lombard: None. **C.S. Wood:** None. **L. Wilson:** None. **S.K. Wood:** None.

Nanosymposium

490. Psychosocial Stress and the Brain

Location: 140A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 490.02

Topic: E.05. Stress and the Brain

Support: MH 093981

5-T32-MH-014654-35

NARSAD 17830

AHA 14BGIA14370026

Title: Distinct adaptations of locus coeruleus neurons to repeated social stress in adolescent and adult rats

Authors: *G. A. ZITNIK, III[1], A. L. CURTIS[1], S. K. WOOD[2], R. J. VALENTINO[1]; [1]Anesthesiol. and Critical Care, Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract: Stress is a prevalent societal issue linked to the development of several psychological disorders. Accumulating evidence suggests that adolescence represents a sensitive period during

which chronic stress may reorganize brain circuits, influencing behavior and susceptibility to certain stress-related disorders. Moreover, there appears to be age-related differences in the ability to cope with chronic stress, although the underlying mechanisms remain unknown. Critical for the acute response to stress, as well as the adaptation to chronic stress, is the locus coeruleus-norepinephrine (LC-NE) system. The present study compared LC neuronal discharge in adolescent vs. adult male rats during exposure to social stress. Adolescent (PND 42) and adult (PND 63) rats were exposed to the resident-intruder model of social stress for five consecutive days, or a control manipulation that involved exposure to a novel cage. LC activity was recorded directly before and after social stress exposure on days 1 and 5. On day 1, tonic LC discharge was significantly increased from baseline in both age groups immediately following stressor exposure. Notably, on day 5, baseline LC discharge of adolescent rats was significantly elevated compared to their discharge rates on day 1 and were similar to rates seen after exposure to stress on day 1. Stress exposure on day 5 had no further effect in these rats. In contrast, baseline LC discharge rates of adult rats were decreased compared to day 1, indicative of adaptation, with tonic LC discharge increased following social stress. Sensory-evoked responses in the LC were also differentially modulated between age groups, with a decrease in auditory-evoked responses post-social stress on day 1 and day 5 among adolescent rats, while responses among adult animals were unchanged. These results suggest that whereas LC neurons of adult rats adapt to excitation associated with social stress, these adaptive mechanisms have not yet developed in adolescence. Previous studies from our group have implicated endogenous opioid afferents to the LC in restraining LC activation from repeated social stress in adult rats. The present findings suggest that this counter-regulatory system may not yet be fully developed in adolescence, making the LC-NE system more vulnerable to excitation by repeated social stress. This sustained increased tonic LC activity may underlie cognitive and behavioral symptoms of stress-related disorders before adulthood. Furthermore, these cellular effects may predispose individuals with a history of repeated social stress during adolescence to psychiatric illnesses.

Disclosures: **G.A. Zitnik:** None. **A.L. Curtis:** None. **S.K. Wood:** None. **R.J. Valentino:** None.

Nanosymposium

490. Psychosocial Stress and the Brain

Location: 140A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 490.03

Topic: E.05. Stress and the Brain

Support: FAPEMIG

CNPQ

CAPES

UFOP

Title: Brain temperature and heart rate increases are correlated with the activation of neurons within the basolateral amygdala in a model of social stress

Authors: *R. C. MENEZES, S. HAIN, D. A. CHIANCA JR, A. R. ABREU;
Depart. Biol. Sci. (DECBI), ICEB, Federal Univ. of Ouro Preto- UFOP, Ouro Preto, Brazil

Abstract: Body temperature increases when individuals experience emotionally significant events. Researchers suggested that the brain temperature increase is a mere result of increase of brain metabolism. However, recent data has pointed out to a mechanism where the heat produced by the brown adipose tissue is essential to lead to increase of brain temperature in a model of social stress. In several stress paradigms increase in body temperature is accompanied by tachycardia. In the present study, we measured the brain temperature (BT) and heart rate (HR) to determine the correlation between both processes in an intruder Wistar rat suddenly introduced into the cage of a resident rat, confined to a small cage, for 20 min. We also evaluated the neuronal activation, determined by the expression of the c-fos protein, in neurons that contain TRPV4 receptors, in the basolateral amygdala (BLA) and in the piriform cortex areas frequently associated with stress responses. Male Wistar rats (300 ± 20g) were anesthetized with ketamine-xylazine (80 mg/kg - 11.5 mg/kg, i.m.) to implant a thermistor below the skull to measure BT and two electrodes to measure ECG. Experiments were performed 7 days later. At the end of the procedures the rats were killed, and their brain were removed for latter evaluation of Fos and TRPV4 expression. Introduction of the intruder rat in the resident's cage promptly increased BT (+1.1±0.1°C vs +0.1± in the control situation, p=0.0003 by unpaired Student t-Test; n=4) and HR (+158±17 bpm vs +9±7 in the control situation, p=0.0003; n=4) in the intruder rat, with the increases in BT and HR been highly correlated (r^2= 0.85, p=0.0007). The intruder rats also showed an increased neuronal activity, demostrated by the increase in the expression of the Fos in the BLA(19±2 per mm^2 vs 4±2 per mm^2 in unstressed animals, p=0.0028 by unpaired Student t-Test; n=4) and in the piriform cortex (48±15 per mm^2 vs 11±5 per mm^2 in unstressed animals, p=0.049; n=4), regions involved with emotional control. Importantly the increase in Fos expression within the BLA was correlated with increases in BT (r^2= 0.65, p=0.007) and HR (r^2= 0.73, p=0.003). Moreover, we have observed the expression of TRPV4 receptors in neurons around the BLA (87±28 per mm^2 vs 58±14 per mm^2 in unstressed animals, p=0.38). Interesting, we also observed the expression of the c-fos protein, in neurons that contain TRPV4 receptors in stressed animals (9±3 per mm^2 vs 1±1 per mm^2 in unstressed animals, p=0.049 by unpaired Student t-Test; n=3). Our results demonstrate that the intruder social stress induces increases in BT and HR, which are correlated with the increased neuronal activity within the BLA.

Disclosures: **R.C. Menezes:** None. **S. Hain:** None. **D.A. Chianca Jr:** None. **A.R. Abreu:** None.

Nanosymposium

490. Psychosocial Stress and the Brain

Location: 140A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 490.04

Topic: E.05. Stress and the Brain

Support: NIH T32MH067533

NIH R01MH085646

NIH R01DA027680

Title: Stress responses, cardiovascular comobidity and functional impairment in schizophrenia

Authors: ***K. L. NUGENT**[1], J. LIU[1], B. DAVIS[1], J. CHIAPPELLI[1], S. DAUGHTERS[2], L. E. HONG[1];
[1]Psychiatry, Maryland Psychiatric Res. Ctr., Baltimore, MD; [2]Psychology, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: The etiopathophysiology of both schizophrenia (SZ) and cardiovascular disease (CVD) are associated with abnormal stress responses. Persons with SZ die over 20 years younger than age matched peers, with CVD as the leading cause of death. We hypothesized that abnormal stress responses in SZ may be responsible for the high CVD morbidity. Using a lab-based paradigm to measure acute stress response, and allostatic load to measure chronic cumulative stress, we examined contributions of these stress indices on CVD risk in SZ patients (n=20) compared with controls (n=16). The computer based psychological stressor identified individuals who quit during the stress test (distress intolerant; DI) vs. those who did not quit (non-DI). Thirteen biological measures were collected to determine allostatic load (AL), including: overnight 12-hour urine cortisol, epinephrine, and norepinephrine; fasting morning blood DHEA, BMI, waist-hip ratio; fasting HDL and total cholesterol; resting blood pressure and resting heart rate; glycosylated hemoglobin A1C; C-reactive protein. AL score and Framingham Heart Study sex-specific CVD risk score were the primary dependent measures. DI vs. non-DI did not significantly separate the CVD risk in HC ($p=0.416$). However, SZ with DI had significantly greater CVD risk than SZ without DI ($p=0.050$), resulting in a significant group by DI interaction

(p=0.047, adjusted for age). We also found CVD risk score to significantly mediate the association between DI and community-based functional capacity as measured by the UPSA-2, explaining 23% of the effect (CI:13-89%). These results suggested that a maladaptive acute stress response may be associated with increased CVD risk in SZ, and that this path is partially responsible for functional impairments. We found SZ to have significantly higher AL (mean(SD)=3.9(2.7)) as compared to controls (mean(SD)=2.6(1.9), p=0.05). Greater AL, indicating increased bodily wear and tear, was significantly associated with reduced functional capacity in SZ (β=-3.36, p=0.004). We then investigated the relationship between CVD risk and AL, by removing the redundant measures from the calculation of AL. We found that in SZ, greater AL was significantly associated with increased CVD risk (B=0.140, p=0.027). These results showed for the first time that cumulative stress load is increased in SZ as compared to age matched controls, and that AL may be an important determinant of impaired functional capacity and CVD risk. Specific targeted treatments for modification of acute stress responses and chronic biological stress accumulation may reduce CVD risk and functional impairments in this population.

Disclosures: K.L. Nugent: None. **J. Liu:** None. **B. Davis:** None. **J. Chiappelli:** None. **S. Daughters:** None. **L.E. Hong:** None.

Nanosymposium

490. Psychosocial Stress and the Brain

Location: 140A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 490.05

Topic: E.05. Stress and the Brain

Support: DA09082

MH040008

Title: Social stress engages amygdalar corticotropin releasing factor and brainstem enkephalinergic afferents to the rat locus coeruleus

Authors: *B. A. REYES[1], G. A. ZITNIK[2], C. E. FOSTER[1], R. J. VALENTINO[2], E. J. VAN BOCKSTAELE[1];
[1]Pharmacol. & Physiol., Drexel Univ. Col. of Med., PHILADELPHIA, PA; [2]Anesthesiol. and Critical Care Med., Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Stress exposure promotes the development of diverse psychopathological disorders. Stress activates the locus coeruleus (LC), a nucleus that sends projections throughout the neuraxis and receives afferents from multiple brain regions including the central nucleus of the amygdala (CeA) and the nucleus paragigantocellularis (PGi). Previous electrophysiological studies from our laboratory demonstrated that with repeated social stress the LC becomes regulated by corticotropin-releasing factor (CRF) and endogenous opioids. Using a rodent model of social stress, the present study was designed to identify the neural circuitry that activates the LC following social defeat. Male Sprague-Dawley rats were injected with the retrograde tracer, Fluorogold (FG) into the LC. Three days following FG injection, rats were subjected to repeated (5 days) social defeat or control manipulation and perfused 90 minuttes after the last session. Sections through the lower brain stem and forebrain were collected and processed for immunocytochemical detection of c-Fos, a marker of neuronal activity, FG and CRF or enkephalin (ENK). Consistent with our previous tracing studies, retrogradely labeled neurons from the LC were distributed throughout the rostro-caudal segments of the CeA and PGi. Cell counts revealed that c-Fos expression in these regions was significantly increased in the social stress group ($P < 0.05$) compared to control group. Nearly 80% of c-Fos expressing neurons in the CeA and PGi were retrogradely labeled from the LC. Approximately 74% of c-Fos expressing neurons in the CeA that were retrogradely labeled showed CRF-immunoreactivity while approximately 50% of c-Fos expressing neurons in the PGi that were retrogradely labeled showed ENK immunoreactivity. These results indicate that social stress activates neurochemically distinct neuronal populations in the CeA and PGi that may in turn drive CRF and ENK release in the LC resulting in cellular adaptations that have important consequences on behavioral and physiological responses to stress.

Disclosures: B.A. Reyes: None. **G.A. Zitnik:** None. **C.E. Foster:** None. **R.J. Valentino:** None. **E.J. Van Bockstaele:** None.

Nanosymposium

490. Psychosocial Stress and the Brain

Location: 140A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 490.06

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant MH79201

NIH Grant MH60451

NIH Grant MH093092

Title: Brain serotonin deficiency exacerbates social avoidance behavior and impairs antidepressant-like responses to fluoxetine following psychosocial stress

Authors: *B. D. SACHS, J. R. NI, M. G. CARON;
Duke Univ., DURHAM, NC

Abstract: More than one in ten Americans over the age of twelve are currently taking antidepressant medications, most commonly for the treatment of major depression or anxiety disorders. Unfortunately, antidepressant drugs fail to achieve remission in as many as 60% of patients, thus leaving millions of individuals in need of improved therapies. However, the biological basis of antidepressant responses remains unknown, and the factors that lead to the development of depression and anxiety disorders have not been conclusively identified. Serotonin (5-HT) is thought to play a key role in the etiology and treatment of depression and anxiety, but whether 5-HT deficiency increases susceptibility to depression- and anxiety-like behavior induced by psychosocial stress has not been established. Similarly, whether 5-HT deficiency impacts the ability of antidepressants to reverse stress-induced phenotypes has not been explored. Here, we compared behavioral responses to psychosocial stress and chronic fluoxetine (FLX) administration in wild-type and tryptophan hydroxylase 2 (R439H) knock-in (Tph2KI) mice, which harbor a partial loss-of-function mutation in the 5-HT synthesis enzyme, Tph2. In addition, we explored the potential of using designer receptors exclusively activated by designer drugs (DREADDs) to achieve antidepressant-like effects via the inhibition of the lateral habenula (LHb) following psychosocial stress. Our results demonstrate that 5-HT deficiency leads to increased susceptibility to psychosocial stress and that chronic FLX administration fails to reverse stress-induced social avoidance in Tph2KI mice, despite reversing this phenotype in wild-type animals. In contrast, inhibition of LHb activity using DREADDs is sufficient to restore social behavior in both WT and Tph2KI mice, suggesting that LHb inhibition may represent an attractive approach to induce antidepressant responses even in populations that fail to respond to selective serotonin reuptake inhibitors.

Disclosures: B.D. Sachs: None. **M.G. Caron:** None. **J.R. Ni:** None.

Nanosymposium

490. Psychosocial Stress and the Brain

Location: 140A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 490.07

Topic: E.05. Stress and the Brain

Support: MH049698 (JPH)

MH069860 (JPH)

MH097430 (JMM)

AHA Postdoctoral Fellowship (BM)

Title: Chronic stress promotes inhibition of medial prefrontal cortex output neurons

Authors: *J. M. MCKLVEEN[1,2], J. R. SCHEIMANN[1,2], R. L. MORANO[1], S. N. CASSELLA[2,3], S. GHOSAL[1,2], B. MYERS[1], M. L. BACCEI[2,4], J. P. HERMAN[1,2]; [1]Psychiatry and Behavioral Neurosci., [2]Neurosci. Grad. Program, [3]Neurol., [4]Anesthesiol., Univ. of Cincinnati, Cincinnati, OH

Abstract: Multiple neuropsychiatric disorders, e.g. depression and posttraumatic stress disorder, are linked to dysfunctional stress regulation and glucocorticoid dyshomeostasis. Notably, chronic stress decreases pyramidal neuronal dendritic complexity and increases interneuronal arborization in the medial prefrontal cortex (mPFC), a known stress-regulatory region. The functional consequences of these morphological changes are currently unknown. Presently, we used physiological, neuroanatomical, and behavioral techniques to test the overall hypothesis that chronic stress inhibits mPFC output neurons, ultimately affecting behavior. Adult male rats (n=5 per group) underwent two weeks of chronic variable stress (CVS) or were unhandled (naïve), beginning at ~P60. Miniature inhibitory and excitatory postsynaptic currents (mIPSCs and mEPSCs) were recorded from the same layer V pyramidal neurons in the infralimbic mPFC (n=21-25 cells per group). Chronic stress selectively increased mIPSC frequency and the ratio of mIPSC to mEPSC frequency, with no effect on mIPSC amplitude. There were no significant differences in mEPSC frequency or amplitude between chronically stressed and naïve rats. The data suggest that chronic stress increases inhibition of infralimbic neurons via presynaptic mechanisms involving enhanced GABA release. Anatomical analysis, from a separate cohort of animals, indicated that chronic stress decreased glucocorticoid receptor (GR) expression specifically in GAD67-positive neurons (and not glutamatergic neurons), suggesting that increased inhibitory tone in the mPFC following chronic stress may be due to loss of a GR-mediated brake on interneuronal activity. Furthermore, inhibitory (GAD65-positive) appositions onto glutamatergic (CaMKIIalpha-positive) cells were increased by chronic stress, suggesting that chronic stress also increases inhibitory innervation of the glutamatergic output neurons. Using the delayed spatial win-shift (DSWS) paradigm, we tested the hypothesis that chronic stress impairs behavioral flexibility, a key mPFC-mediated behavior. During chronic stress, rats initially made significantly more errors in the DSWS, but eventually acquired the task. Taken together, the data suggest that chronic stress may take prefrontal glutamatergic output neurons 'offline', limiting the ability of the mPFC to promote behavioral flexibility and adaptation.

Understanding the mechanisms by which chronic stress affects mPFC function is critical for effective therapeutic development for stress-related disorders.

Disclosures: **J.M. McKlveen:** None. **J.R. Scheimann:** None. **R.L. Morano:** None. **S. Ghosal:** None. **B. Myers:** None. **M.L. Baccei:** None. **J.P. Herman:** None. **S.N. Cassella:** None.

Nanosymposium

490. Psychosocial Stress and the Brain

Location: 140A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 490.08

Topic: E.05. Stress and the Brain

Support: NIH(RO1MH098348)

Title: Neuroendocrine response to psychosocial stress

Authors: ***M. D. WHEELOCK**, N. G. HARNETT, K. H. WOOD, S. MRUG, D. C. KNIGHT; UAB, Birmingham, AL

Abstract: Introduction. Prior work has demonstrated negative affectivity (e.g. anxiety level) alters PFC-amygdala connectivity (Etkin and Wager, 2007, Hahn et al., 2011, Kim et al., 2011, Rabinak et al., 2011, Roy et al., 2013). Prior work has also demonstrated that stressful tasks elicit brain activation changes within the same PFC-amygdala network (Pruessner et al., 2008, Dedovic et al., 2009, Jones et al., 2011, Kazen et al., 2012, Mizrahi et al., 2013). The present study investigated the relationship between negative affectivity, cortisol release, and brain activation in response to a stressful task. **Experimental Design.** Blood oxygen level dependent (BOLD) fMRI, stress response (indexed via cortisol), and negative affectivity (i.e. State-Trait Anxiety Inventory; Spielberger, 1983) were collected from participants completing the Montreal Imaging Stress Task (MIST) (Dedovic et al., 2005). **Results.** Functional MRI data demonstrated differential activation of dmPFC, vmPFC, and amygdala under Stress versus No-Stress conditions during the MIST. During the Stress scan, participants showed greater BOLD signal activation in the dmPFC, whereas greater deactivation was observed within the vmPFC and amygdala. Salivary cortisol data was collected prior to and following the stress task. There was a significant increase in the baseline (pre-task) to peak cortisol (20 minutes post-task) response. Cortisol increase from baseline was used in a linear regression with fMRI data collected during stress exposure. Activity within the vmPFC varied with cortisol release such that as cortisol increased, differential (i.e. Stress minus No-Stress MIST) vmPFC activity decreased. Negative

affectivity (Trait anxiety) was measured prior to the MIST. Scores from the trait anxiety scale were used in a linear regression with fMRI data collected during the MIST. Activity within the dmPFC varied with trait anxiety score such that as trait anxiety increased, differential (i.e. Stress minus No-Stress MIST) dmPFC activity increased. **Conclusion**: Negative affectivity influences brain activity during stress exposure. Individuals with decreased vmPFC activity have greater cortisol release in response to stress. This research will elucidate the mechanisms by which negative affectivity (anxiety) influences the neuroendocrine response to psychosocial stress.

Disclosures: M.D. Wheelock: None. **N.G. Harnett:** None. **K.H. Wood:** None. **S. Mrug:** None. **D.C. Knight:** None.

Nanosymposium

490. Psychosocial Stress and the Brain

Location: 140A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 490.09

Topic: E.05. Stress and the Brain

Support: NIH Grant R01MH093473

NIH Grant R01MH097243

Title: Repeated social stress-induced modification of innate immunity was prevented by glucocorticoid receptor antagonism

Authors: *S. H. JUNG[1,2], Y. WANG[1], B. READER[1], J. F. SHERIDAN[1,2];
[1]Inst. for Behavioral Med. Res., Wexner Med. Center, Ohio State Univ., Columbus, OH; [2]Div. of Biosciences, Col. of Dent., Ohio State Univ., Columbus, OH

Abstract: Socially stressed individuals are less healthy psychologically and physiologically, and a concrete body of evidence shows that people under chronic social stress are more vulnerable to the development of psychological disorders and more susceptible to infectious challenges. Previous studies from our lab have shown that repeated social disruption (RSD), a rodent model of chronic social stress, altered innate immune system responses, including recruitment of bone marrow-derived monocytes to the brain and the induction of glucocorticoid insensitivity in splenic macrophages. Because of our recent findings showing that RSD-induced changes in the innate immunity are associated with glucocorticoid (GC) regulation, and a recent paper showing potential effects of blocking GC receptor on restraint-induced modification of rodent behavior,

we developed and tested our hypothesis that blocking systemic GC receptor would prevent RSD-induced changes in the intracellular modification in splenic macrophages. As previously described, 6 cycles of RSD were given for 2 hours (5pm - 7pm) to C57BL/6 male mice in RSD groups. RU486 (25mg/kg/day) was daily dissolved in polyethylene glycol (PEG) and then subcutaneously injected at 4 pm from 3 day before stress was given and during the stress days. Bone marrow and spleens were collected in the morning after the last RSD cycle. The data showed that RSD induced a shift in hematopoiesis favoring myelopoiesis, i.e., a decrease in the lymphoid population and an increase in the myeloid population in bone marrow. RU486 treatment blocked the bone marrow changes. RSD has been shown to induce an increase in both $CD11b^{+}$, $Ly6C^{Intermediate}$ and $CD11b^{+}$, $Ly6C^{High}$ splenocyte populations. RU486 treatment blocked the RSD-induced enhancement in the CD11b+ populations in spleen. Data from intracellular molecular analysis showed that RSD increased CCL2 mRNA expression in splenic macrophages, increased the mRNA ratio of fkbp51to fkbp52, but decreased GC receptor mRNA expression in splenic macrophages. However, these RSD-induced changes were prevented by RU486 treatment. In conclusion, this study showed that RSD-induced modification of innate immunity that were prevented by blocking systemic glucocorticoid receptors. This research was supported by NIH grants R01MH093473 and R01MH097243 to Dr. John F. Sheridan

Disclosures: **S.H. Jung:** None. **Y. Wang:** None. **B. Reader:** None. **J.F. Sheridan:** None.

Nanosymposium

490. Psychosocial Stress and the Brain

Location: 140A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 490.10

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: European Research Council (grant #281338, GxE molmech

Behrens Weise Stiftung

MH071538

MH58922

Title: Gene by environment interaction on post-traumatic stress disorder. Role of genetic and epigenetic differences in FKBP5

Authors: *T. KLENGEL[1,2], S. ROEH[2], M. REX-HAFFNER[2], B. BRADLEY[1], K. J. RESSLER[1], E. B. BINDER[2,1];
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Abstract: The majority of psychiatric disorders result from an interaction of the individual's genetic predisposition and environmental factors to a varying degree. We previously showed a molecular epigenetic mechanism for the interaction of rs1360780 in the GR-regulating co-chaperone FKBP5 and childhood trauma on the development of post-traumatic stress disorder (PTSD) (Klengel et al., Nat Neurosci 2013). Here we describe the additional interaction of a large structural variation in FKBP5 with childhood abuse on the risk for psychiatric symptoms in adulthood. This structural variant strongly modulates the previously described interaction and further refines the interplay of FKBP5 with childhood abuse on the risk for psychiatric disorders. FKBP5 rs1360780 interact with child abuse exposure (CTQ) on the development of current PTSD symptoms (mPSS) in adulthood ($F_{1963,2} = 4.40$, $P = 0.012$) and on lifetime PTSD risk (CAPS) (risk allele carrier: $X2 = 28.6$, $df = 2$, $P < 0.001$, carriers of the protective genotype: $X2 = 2.02$, $df = 2$, $P = 0.36$). Exposure to childhood trauma lead to a significant DNA demethylation of CpGs around glucocorticoid responsive elements (GREs) of FKBP5 in abused individuals only in carriers of the risk allele. This emphasizes the effects of early trauma severity on FKBP5 demethylation in a genotype dependent way (Klengel et al., Nat. Neurosci. 2013). Using a next generation sequencing approach we identified a 3.3kb long insertion that occurred in 21.3 % of individuals (N=413) and consisted of LINE and SINE element sequences. The insertion only occurred on the previously reported risk haplotype tagged by rs1360780. When testing the insertion for interaction with child abuse on predicting current and lifetime PTSD, as well as depressive symptoms, we observed a significant interaction, with the presence of insertion having protective effects (pint=0.0004 on mPSS, N=231; pint<0.00001 on BDI, N=232). Individuals carrying this insertion on the risk haplotype background were protected from the increase in risk, suggesting a complex interplay of common and structural variants in gene environment interactions of the FKBP5 locus. The insertion contains LINE and SINE elements and may have arisen from retrotransposon insertion. The presence of this structural variant leads to altered DNA methylation and gene expression profiles locally but also in more proximal loci. These data underline the importance of structural variants for GxE interaction analyses in interplay with common single nucleotide variants. Insertion of repetitive sequences may change the 3D organization, DNA methylation and transcriptional responsiveness of the distal and proximal loci.

Disclosures: T. Klengel: None. **S. Roeh:** None. **M. Rex-Haffner:** None. **B. Bradley:** None. **K.J. Ressler:** None. **E.B. Binder:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Max Planck Institute of Psychiatry.

Nanosymposium

490. Psychosocial Stress and the Brain

Location: 140A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 490.11

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Molecular markers linking the experience of an adverse childhood with the development of depression in adulthood: Focus on SIRT1

Authors: *L. LO IACONO[1], F. VISCO-COMANDINI[2], A. VALZANIA[1], M. COVIELLO[4], L. ROSCINI[3], P. SESTILI[5], A. TROISI[4], S. PUGLISI-ALLEGRA[1,3], V. CAROLA[1];
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[4]Systems medicine, Univ. Tor Vergata, Rome, Italy; [5]Inst. Superiore di Sanità, Rome, Italy

Abstract: The exposure to an adverse childhood is considered a risk factor for Major Depression (MD) in clinical population. Patients with a history of traumatic childhood show a subtype of depression characterized by earlier onset, poor treatment response and more severe symptoms (Miniati et al., J Psych Res 2010) likely dependent on specific etiological pathways. The long-lasting molecular mechanisms engaged during early traumatic events and that determine a risk for depression, are poorly understood and their exploration would require validated preclinical models. Moreover, no specific biomarkers up to date are known to support diagnosis. In this study, we have developed a preclinical model of early stress-induced adult depression by applying a social isolation stress (ESI) in pre-adolescent (P14-21) mice. Pre-adolescence is a critical developmental period when the maturation of visual and motor abilities accompanies the first elements of social play (Pellis and Pasztor, Dev Psychobiology 1999). Moreover the strong neuronal plasticity and the active epigenetic machinery cooperate during this period to translate environmental inputs into long-lasting gene expression changes (Lister et al., Science 2013). Our results show that ESI mice develop adult depression-like phenotype, social avoidance, and anhedonia. In order to explore the role of epigenetic modifications in the long-term effects of early environmental experiences, we have analysed the level of expression of 40 genes involved in epigenetic mechanisms in ESI and unhandled adult mice. We have identified a number of markers of the early stress in the brain, including transcripts for NPY, DRD2, Camk2a, HDAC1 and HDAC6, SIRT6 and SIRT1. Interestingly SIRT1 mRNA was strongly reduced in the brain as well as in Peripheral Blood Mononuclear Cells (PBMC) of adult mice, and its level of expression, measured few days before the behavioral tests, was able to predict the depression-like phenotype. When SIRT1 transcript was measured at the end of ESI stress, an increased expression was instead observed, suggesting that a transient induction in response to the stress

precedes a long-term decrease of its expression. Finally we conducted a pilot study in human subjects that had been diagnosed for MD. We found that their blood level of SIRT1 expression significantly correlated with their depression scores only when this was associated with low, but not adequate parental care during their infancy. This study proposes SIRT1 as an important developmental mediator translating childhood psychosocial stress into biological risk for depression.

Disclosures: **L. Lo Iacono:** None. **F. Visco-Comandini:** None. **A. Valzania:** None. **M. Coviello:** None. **L. Roscini:** None. **P. Sestili:** None. **A. Troisi:** None. **S. Puglisi-Allegra:** None. **V. Carola:** None.

Nanosymposium

490. Psychosocial Stress and the Brain

Location: 140A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:00 AM

Presentation Number: 490.12

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: The impact of transcranial direct current stimulation (tDCS) on resilience, stress, compassion fatigue and empathic response in professional nurses

Authors: *M. STANTON[1], R. HAUSER[2];
[2]Educ., [1]Univ. of Alabama, Tuscaloosa, AL

Abstract: The purpose of the study is to determine the effect of transcranial direct current stimulation (tDCS) on measured levels of resilience, and empathy in professional nurses who experience compassion fatigue and stress. The literature has determined relationships between lowered levels of resilience, stress, compassion fatigue and decreased empathy. Enhanced levels of resilience appear to improve empathic responses and overall emotional well-being. Nurses are prone to compassion fatigue or secondary traumatic stress because of their work in high stress environments and the presence of compassion fatigue and or stress sequelae. Stress reactions have been related in research to decreased resilience. The use of tDCS may be a potential strategy for improving resilience and eliminating chronic stress responses in affected nurses. Although there has been little or no research examining the impact of tDCS on resilience, compassion fatigue stress sequelae and empathic response in nurses, there are a number of studies that have examined the effects of tDCS on post-traumatic stress disorder predominantly in military veterans. There are also a number of studies that have examined the relationship between tDCS and select emotional responses. The specific aims of this research were to

determine: 1.0 If there is a difference in the level of compassion fatigue, stress, resilience and empathy based on the tDCS amperage that is delivered. 2.0 If there are statistically significant differences before and after the administration of tDCS on levels of compassion fatigue, stress, resilience and empathic responses for individuals. Subjects were nurses who have worked in busy, stressful health care environments. The reason for using these participants are that they are most vulnerable to compassion fatigue and/or secondary stress symptoms and may have compromised levels of resilience and empathy A flier was provided to registered nurses which provided a brief overview and instructed potential subjects about how to contact the investigator by email or by phone. A timed series counterbalanced research design was used. There were three experimental conditions used in the study. Experimental condition A involved the use of stimulation at 1.0 mA current for 20 minutes, condition B 1.5 mA current for 20 minutes and condition C, 2 mA for 20 minutes. Preliminary results have demonstrated that that tDCS has a positive impact on resilience and appears to mitigate compassion fatigue.

Disclosures: M. Stanton: None. **R. Hauser:** None.

Nanosymposium

491. Perception and Imagery

Location: 147A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 491.01

Topic: F.01. Human Cognition and Behavior

Support: William Hewlett Stanford Graduate Fellowship

NRSA Grant NEIF32EY019815

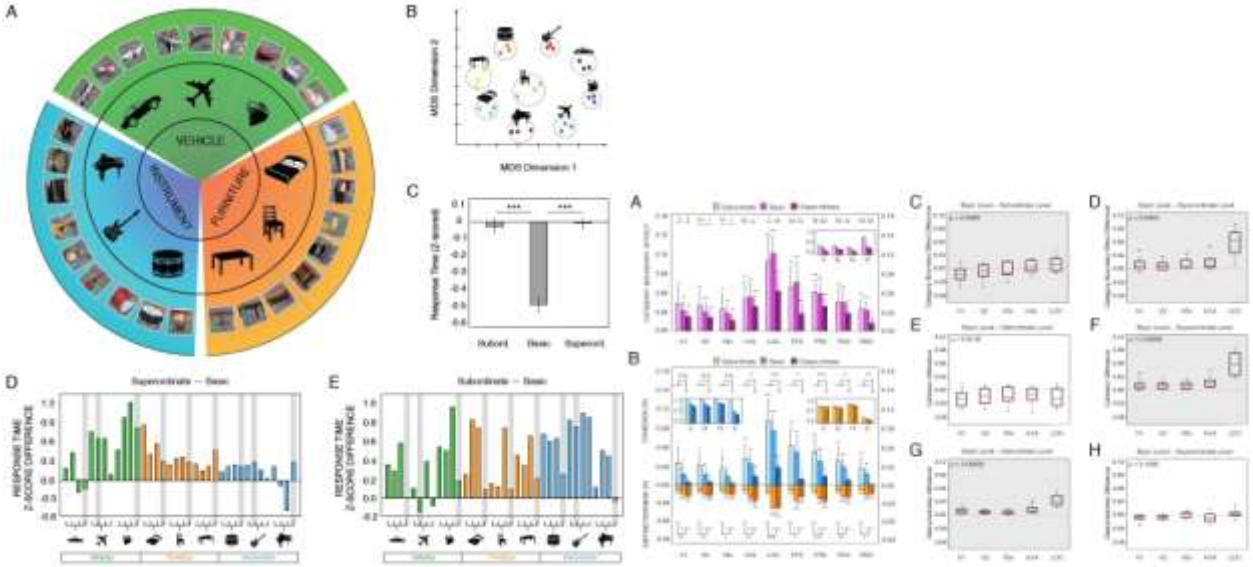
NIH Grant 1 R01 EY019429

Title: Category cohesion and distinctiveness in human visual cortex favor basic level representations

Authors: *M. IORDAN[1], M. R. GREENE[1], D. M. BECK[2], L. FEI-FEI[1];
[1]Computer Sci., Stanford Univ., Stanford, CA; [2]Psychology and Beckman Inst., Univ. of Illinois, Urbana-Champaign, Urbana, IL

Abstract: Objects can be simultaneously categorized at multiple levels of specificity ranging from very broad (”natural object”) to very distinct (”Mr. Whiskers”), with a mid-level of generality (basic level: “cat”) often providing the most cognitively useful distinction between

categories. It is unknown, however, how this hierarchical representation is achieved in the brain. Using multi-voxel pattern analyses, we examined how well each taxonomic level (superordinate, basic, and subordinate) of real-world object categories (Fig. 1) is represented across occipito-temporal cortex. We found that although in early visual cortex objects are best represented at the subordinate level (the level at which the objects share the greatest similarity), this advantage diminishes in favor of the basic level as we move up the visual hierarchy, disappearing in object-selective regions of occipito-temporal cortex (LOC) (Fig. 2). This pattern stems from a combined increase in within-category similarity (category cohesion) and between-category dissimilarity (category distinctiveness) of neural activity patterns at the basic level, compared to both subordinate and superordinate levels, suggesting that successive visual areas may be optimizing basic-level representations.



Disclosures: **M. Iordan:** None. **M.R. Greene:** None. **D.M. Beck:** None. **L. Fei-Fei:** None.

Nanosymposium

491. Perception and Imagery

Location: 147A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 491.02

Topic: F.01. Human Cognition and Behavior

Title: Neural discriminability of object features predicts perceptual organization

Authors: *E. J. WARD[1], M. M. CHUN[2];
[2]Psychology, [1]Yale Univ., New Haven, CT

Abstract: What we see is constrained by how our perceptual system partitions the world. Perceptual organization is classically explained by Gestalt principles, like proximity, similarity, and closure. However, we now know that experience, intention, and task can warp the neural representation of visual information across the cortex (Cukur, et al., 2013). Such effects hint that visual representations may be much more flexible, varying from person to person and between tasks. In this study, we measured neural discriminability of object features as way to explore variation in perceptual organization across individual observers. We scanned observers while they viewed simple objects varied along three feature dimensions: shape, color, and texture. Using activity patterns in the inferior intraparietal sulcus, a region implicated in facilitated processing of grouped items (Xu & Chun, 2007), we calculated pattern similarity between patterns evoked by the same feature (e.g. correlating patterns for two circles) and pattern similarity between patterns evoked by different features (e.g. correlating patterns for circles and squares). The difference between these correlations gave a "discriminability index" for each feature. Afterward, observers performed a perceptual grouping task outside the scanner. The Repetition Discrimination Task (Palmer & Beck, 2007) measures perceptual grouping strength between features. Displays contained a single row of items alternating between features (e.g., circle and squares), except for a pair in which the same feature repeated. Observers detected these repetitions, and response time and accuracy were measured for two conditions: In within-group trials, a grouping factor from an orthogonal, task-irrelevant feature dimension (such as color) biased the repeated target pair to be perceived as part of the same perceptual group. In between-group trials, the orthogonal grouping factor biased the target to be perceived as part of two different groups. This task thus produced a "grouping index," where within-group detection was faster than between-group detection. We then correlated the neural data with this independent behavioral measure and found that observers' neural feature discriminability index positively predicted their grouping index: the more distinct the neural feature representations, the stronger the grouping effect ($r(21)=0.51$, $p=0.017$). These results show that neural measures of feature discriminability can be used to predict perceptual grouping of task-relevant and task-irrelevant visual features, suggesting that these measures may track intrinsic differences in perceptual organization across individuals.

Disclosures: E.J. Ward: None. **M.M. Chun:** None.

Nanosymposium

491. Perception and Imagery

Location: 147A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 491.03

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant EY016975

Veterans Administration

Chancellor's Faculty Partnership Fund at UC Berkeley

NEI Core Grant EY003176

Title: Redefining the metric of visual space: The influence of visual field boundaries and attention on crowding performance

Authors: *M. A. SILVER, F. C. FORTENBAUGH, L. C. ROBERTSON;
Univ. of California, BERKELEY, CA

Abstract: Performance on perceptual tasks often varies as a function of visual field location, even for stimuli that have equal eccentricities (in degrees of visual angle). However, the brow and nose cause the visual field to be asymmetrically bounded in humans. How might these boundaries effect representation of location in the visual system? Here we show that a well-studied perceptual asymmetry, the lower visual field advantage in visual crowding, is explained by a new metric of visual space that is based on visual field extent, both at the group and individual level. Subjects performed a crowding task in which they reported the orientation of a grating flanked by four similar gratings and presented either above or below central fixation. We replicated previous reports that participants are better at judging the orientation of the crowded grating along the lower vertical meridian compared to the upper vertical meridian. It has been proposed that this asymmetry is due to differences in the resolution of spatial attention that may facilitate visual search along the ground plane. In contrast, the metric we propose incorporates the fact that humans have smaller upper than lower visual field extents (VFE), and it replaces degrees of visual angle with a distance measure that is relative to an individual's VFE for a given radial direction (percentage of visual field extent: %VFE). We first measured each participant's upper and lower VFE and found that the performance asymmetry in visual crowding was largely accounted for by individual variability in visual field extent. We also found that this performance asymmetry was eliminated when target locations were equated using the new metric (i.e., locations with equal %VFE). Next, we manipulated the degree to which participants were able to voluntarily focus their attention at the target location. We found a significant correlation between individuals' visual field extents and performance asymmetries only when subjects were able to focus endogenous attention at the target location. When targets were presented for brief (150 ms) durations, thereby reducing the ability of participants to direct attention to the target grating, no correlation was observed between visual field extent and crowding performance asymmetry. The

%VFE metric utilized here provides novel insights regarding visual field asymmetries in perception and raises important questions regarding appropriate models for describing the organization of visual field representations in the brain. The results also demonstrate a critical role for endogenous spatial attention in mediating the relationship between visual field shape and performance asymmetries.

Disclosures: **M.A. Silver:** None. **F.C. Fortenbaugh:** None. **L.C. Robertson:** None.

Nanosymposium

491. Perception and Imagery

Location: 147A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 491.04

Topic: F.01. Human Cognition and Behavior

Title: Neural representation of different boundary cues

Authors: ***K. FERRARA**, S. PARK;
Cognitive Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Boundaries are fundamental features that define a scene, contribute to its geometric shape, and restrict our movement within an environment. However, not all boundaries are equally effective for navigation. For example, 4-yr-olds reorient in accord with the geometry of a layout defined by walls that are 2 cm in height (e.g., curb), but fail to do so when the walls are replaced by a flat mat on the floor (Lee & Spelke, 2011). The present research asks whether there exists a neural signature that differentiates between the amount of 3D vertical boundary cue in a scene. We used artificially-created scene stimuli: a Mat condition where no vertical structure is present, a Curb in which there is minimal vertical structure, and a full Wall. Participants viewed images in blocks of 12 seconds while performing a one-back task. We measured univariate and multivoxel pattern activity across two scene-selective regions: parahippocampal place area (PPA) and restrosplenial cortex (RSC). In Experiment 1, univariate activity in PPA showed a stepwise increase from the Mat, to Curb, to Wall. This indicates impressive sensitivity to the presence of the minimal visual cue of the Curb, even though it and the Mat are quite visually similar. In RSC, activity for the Mat and Curb were no different from one another, only the Wall cue had significantly greater activity than the other two. This suggests that RSC may represent the functional affordance of a boundary. Multi-voxel pattern analyses using a linear classifier confirmed the above results. When the stimuli were inverted, thereby erasing the ecological validity of boundaries that extend from the ground up (Exp. 2), the disproportionate sensitivity to

the presence of the Curb in PPA was strongly diminished, suggesting an acute sensitivity to the presence of grounded vertical boundary structure. To further test the hypothesis that RSC may represent the functional affordance of boundaries, we tested RSC's activity for stimuli that incrementally varied in boundary height (Exp. 3). We hypothesized that if RSC's response reflects the functional affordance of a boundary, its univaraite activity would match the point at which people determine that a boundary has changed from an easy-to-cross curb to a difficult-to-cross wall. This is precisely what we found: RSC's response changed radically between height 5 to 6, which was a behavioral categorical decision point for functional affordance—whether the boundary limits the viewer's potential navigation or not. Collectively, this research serves to highlight boundary structure as a key component of space that is represented in qualitatively different ways across two scene-selective brain regions.

Disclosures: K. Ferrara: None. **S. Park:** None.

Nanosymposium

491. Perception and Imagery

Location: 147A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 491.05

Topic: F.01. Human Cognition and Behavior

Support: NIHR01-NS065186

Title: Ventral temporal cortex is a locus of visual object perception

Authors: *K. J. MILLER[1], D. HERMES[2], F. PESTILLI[2], R. P. N. RAO[3], J. G. OJEMANN[3]; [1]Neurosurg., [2]Stanford Univ., Stanford, CA; [3]Univ. of Washington, Seattle, WA

Abstract: Visual perception is the process of combining simple visual features to extract an independent concept, to which memories, expectations, and context designate a larger meaning. Anatomic regions where this process might take place will be revealed if basic physical properties of stimuli are independently represented alongside recognition of abstract conceptual objects. In ventral temporal cortex, there are known to be anatomically distinct regions corresponding to different categories of objects encountered in the world, but it has not been concretely established whether these regions represent accumulating visual evidence for these categories, or whether they reflect the formed percept of each object category. Human ventral temporal neuronal population responses were measured with implanted electrodes during a face and house picture decision task where visual evidence was parametrically degraded. Robust,

category-selective, neurometric functions were found for face stimuli on the fusiform gyrus and house stimuli on the lingual gyrus. The shape of these neurometric functions was robust whether subjects correctly perceived the stimulus type or not. We constructed a basic classifier from a choice-free localizer experiment, and applied it to the decision task. At low levels of stimulus noise, the classifier robustly predicted stimulus the stimulus type. At high levels of noise, the classifier could not predict the stimulus type, but predicted the subjects' choice better than chance. Therefore, ventral temporal cortex must be an active perceptual locus, where simple features and abstract recognition converge.

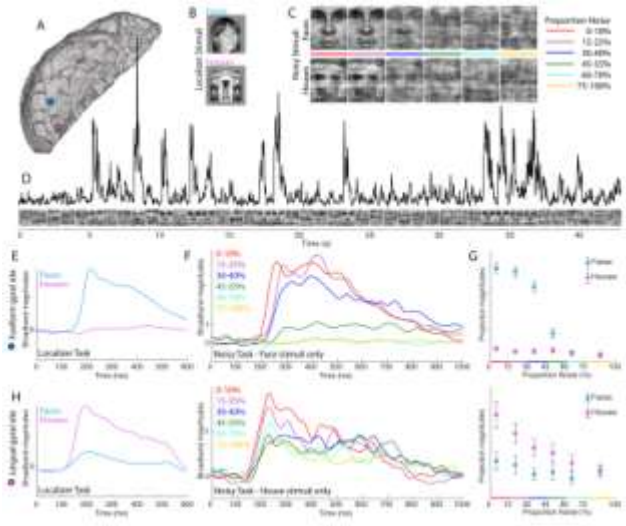


Fig: Experimental setting and neural population response. (A) ECoG electrodes: Blue - fusiform; Pink - lingual. (B) Localizer task. (C) Noisy-task (Heekeren, et. al. Nature 2004). (D) The timecourse of broadband spectral change in the electrical potential (blue site), with stimuli. (E) Localizer task, averaged responses (blue site). (F) Noisy task, averaged responses, (faces only). (G) Neurometric function. (H) As in E-G, but for pink site, and house stimuli.

Disclosures: **K.J. Miller:** None. **D. Hermes:** None. **F. Pestilli:** None. **R.P.N. Rao:** None. **J.G. Ojemann:** None.

Nanosymposium

491. Perception and Imagery

Location: 147A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 491.06

Topic: F.01. Human Cognition and Behavior

Support: NWO Veni Grant 451-12-021

NWO Vici Grant 453-09-002

Title: The relation between oscillatory EEG activity and the laminar specific BOLD signal

Authors: *R. SCHEERINGA[1], P. J. KOOPMANS[2], T. VAN MOURIK[1], O. JENSEN[1], D. G. NORRIS[1];
[1]Radboud Univ. Nijmegen, Nijmegen, Netherlands; [2]Univ. of Oxford, FMRIB, Oxford, United Kingdom

Abstract: Recent developments in high-resolution fMRI have made it possible to measure BOLD signals with laminar resolution in humans. The relevance of this technique for neuroscience would be enhanced if the relationship between laminar BOLD signals and electrophysiology could be elucidated. Laminar electrophysiological recordings in animals have indicated that in the early visual cortex gamma band oscillatory activity is predominantly measured in superficial layers (layers II/III/IV), while alpha and beta band activity show a strong presence in deeper layers (V/VI). In a previous experiment we demonstrated in a visual attention task with simultaneously recorded EEG that alpha/beta and gamma band activity independently contribute to respectively a BOLD decrease and increase in early visual cortex. In this work we present data from a very similar visual attention experiment in which we simultaneously measure EEG and laminar specific BOLD signals from V1, V2 and V3. We investigate whether trial-by-trial changes in the power of different EEG frequency bands show different correlation profiles over the cortical layers with the BOLD signal. The task we used robustly shows sustained gamma band increases and alpha and beta band decreases at single subject level, which previous intracranial and MEG studies have localized to the early visual cortices. In addition, the attention modulation in this task causes robust increases in gamma power and decreases in alpha and beta power. The results of the integrated laminar fMRI-EEG analysis indicate that alpha band power shows a significant negative relation with the BOLD signal across all layers, including deep layers in V1, V2 and V3. The result in deeper layers is in line with observations in animals that alpha band oscillations are relatively strong there. The fact that we also find a relation in superficial layers can possibly be related to the direction of the venous blood flow from deep to superficial. In contrast, gamma band power fluctuations show only a positive relation with the BOLD signal from superficial layers and no relation with deep layers in V2 and V3. In V1 a similar but non-significant trend was observed. These findings for the gamma band are also in line with animal electrophysiology. With this experiment we take a first step towards establishing a neurophysiological basis for the application of laminar fMRI in human cognitive and systems neuroscience, and demonstrate a methodology that can potentially link EEG features to laminar specific processes.

Disclosures: **R. Scheeringa:** None. **P.J. Koopmans:** None. **T. Van Mourik:** None. **O. Jensen:** None. **D.G. Norris:** None.

Nanosymposium

491. Perception and Imagery

Location: 147A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 491.07

Topic: F.01. Human Cognition and Behavior

Support: NIH RO1 DC 012511

NSF subcontract 330161-18110-7341

Title: Fronto-temporal cortex encodes the manner of motion during reading

Authors: ***L. C. QUANDT**[1], E. R. CARDILLO[1], A. KRANJEC[2], A. CHATTERJEE[1]; [1]Univ. of Pennsylvania, Philadelphia, PA; [2]Duquesne Univ., Pittsburgh, PA

Abstract: When describing spatial events, dynamic actions can be decomposed into the path of motion (where the entity moves), and the manner of motion (how the entity moves). The processing of path and manner may be instantiated in two different action-processing streams in the human brain, wherein the egocentric dorsal stream processes path-related information, while the allocentric ventral regions process manner information. Previous research has supported this distinction by measuring BOLD activity during the observation of videos showing animate characters in motion (Wu et al., 2008). However, it is unknown whether the distinction between processing path and manner information is also present when reading language describing path and manner information, which is a level of abstraction beyond the perception of visual movement. In the present study, we explored this possibility by using functional magnetic resonance imaging (fMRI) to characterize neural activity when reading phrases that describe spatial events that either described the path of motion (e.g., “through the hedge”) or the manner of motion (e.g., “push the table”). To test for overall differences between path and manner processing, we compared BOLD activity between the two conditions, masked within the areas that were more active for path and manner than for a control condition. The left fusiform gyrus, left inferior frontal gyrus, and left middle frontal gyrus showed greater activity for the manner condition than the path condition. To investigate regions previously implicated in path/manner distinctions (Wu et al., 2008), we conducted three hypothesis-driven ROI analyses, comparing path and manner in the following regions: left posterior middle temporal gyrus (pMTG), left

superior parietal lobule, and left inferior parietal lobule. Of these three ROIs, only the left pMTG showed significantly more activation for manner trials than path, and none were more active for path conditions. Overall, these results suggest that manner of motion, whether spatially or verbally encoded, is preferentially processed in ventral fronto-temporal regions. We anticipate that this finding may relate to a general tendency for the ventro-laterral temporal lobe to be involved in processing allocentric, conceptual information about action attributes.

Disclosures: **L.C. Quandt:** None. **E.R. Cardillo:** None. **A. Kranjec:** None. **A. Chatterjee:** None.

Nanosymposium

491. Perception and Imagery

Location: 147A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 491.08

Topic: F.01. Human Cognition and Behavior

Support: NRF-2011-0025005

NRF-2013S1A5A8025812

Title: Inattention opens door for unconscious processing during continuous flash suppression

Authors: ***M.-S. KANG**[1,2], K. EO[3,4], O. CHA[3,4], S. CHONG[3,4];
[1]Psychology, Sungkyunkwan Univ., Seoul, Korea, Republic of; [2]IBS Ctr. for Neurosci. Imaging Res., Inst. for Basic Sci., Daejeon, Korea, Republic of; [3]Grad. Program in Cognitive Sci., [4]Psychology, Yonsei Univ., Seoul, Korea, Republic of

Abstract: RATIONALE: Growing evidence indicates that our brain performs semantic analysis for the consciously unavailable stimuli presented due to continuous flash suppression. In the present study, rather counterintuitively, we hypothesized inattention facilitates the extent to which the suppressed stimulus is semantically processed based on the previous findings. First, previous studies indicate that invisible stimuli due to inattention are processed (Luck et al., 1996; Giesbrecht et al., 2007). Second, when attention was diverted, rivalry suppression was attenuated (Zhang et al., 2009; Brascamp et al., 2012). Third, the location of the suppressed stimuli was uncertain in those studies showing semantic processing of the invisible stimulus induced by the continuous flash suppression (Costello et al., 2009; but see Kang et al., 2011). Taken together, if the locus of attention is different from the location of the suppressed stimulus due to its position

uncertainty, it can attenuate rivalry suppression. Thus, inattention to the location of the suppressed stimulus opens door for high-level analysis in the absence of awareness. METHOD: We manipulated attention by adopting a cueing paradigm while measuring the N400 component, a sensitive, electrophysiological index for semantic analysis. Specifically, participants performed a related and unrelated semantic judgment task for the sequentially presented pairs of words. In the in-attention condition, the target word was rendered invisible by the continuous flash suppression and presented in the cued location. In the out-attention condition, the target word was rendered invisible and presented in the uncued location. In the dioptic condition, the target word was presented dioptically on the continuous flash suppression stimuli. RESULT: In the dioptic condition, the N400 was robustly produced with a high semantic judgment performance. Critically, despite the chance level semantic judgment performance, a significant N400 was found in the out-attention condition while the N400 was not found in the in-attention condition. In particular, the magnitude of the N400 of the out-attention condition was about a half of its magnitude obtained from the dioptic condition. CONCLUSION: This result demonstrates that lack of attention is critical for unconscious semantic processing for the suppressed stimuli induced by the continuous flash suppression.

Disclosures: **M. Kang:** None. **K. Eo:** None. **O. Cha:** None. **S. Chong:** None.

Nanosymposium

491. Perception and Imagery

Location: 147A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 491.09

Topic: F.01. Human Cognition and Behavior

Title: Relationship between mu rhythm oscillations and n400 response to incongruent actions

Authors: *R. D. SOOHOO[1], E. VAN[2], J. A. PINEDA[3];
[1]Univ. of California San Diego, Sacramento, CA; [3]Cognitive Sci., [2]Univ. of California San Diego, San Diego, CA

Abstract: Objectives Research shows that activity in the human sensorimotor cortex is modulated by actions as well as the observation of actions, suggesting a role for the Mirror Neuron System (MNS). Activity in the MNS has been attributed to many high level functions including language processing, though there is a lack of research into the extent of this involvement. This study was designed to examine the hypothesis that sensorimotor cortex is involved in processing semantically congruent and incongruent actions by comparing the

rolandic EEG mu rhythm, with known sources in sensorimotor cortex, to a well known indicator of semantic understanding, the N400 event related potential. Methods Thirty-five subjects were run with a thirty-two channel EEG electrocap. Subjects observed 30 trials from a computer monitor, consisting of four static images depicting an action sequence. Images were displayed one at a time for 1.5s each. The final image of each sequence ended the action in either an expected or an unexpected fashion. After each trial subjects responded with a button press whether the action did or did not make sense. Results •Greater N400 negativity for unexpected conditions compared to expected conditions in both C3 and C4 $F(5,165)=2.4$ $p<.05$, with a greater effect over left hemisphere $F(5,165) = 3.79$ $p <.05$ •Greater mu power for unexpected conditions compared to expected conditions in both C3 and C4 $F(1,33) = 93.2$ $p<.001$, with a greater effect over left hemisphere $F(1,33) = 18.3$ $p <.001$. •Greatest N400 effect found within 350-450 ms time span $F(5,165) = 13.82$ $p<.001$ •Greatest mu power change between conditions within 300-600 ms time span $F(1,165) =9033$ $p <.001$ •Correlations between C4 N400 effect and C3 mu power change occurred in both congruent ($r=.38$ $p <.0001$) and incongruent ($r=.3$ $p<.0001$) conditions •Correlations between C4 N400 effect and C4 mu power change only occurred in congruent conditions ($r=.43$ $p<.001$) Conclusions This study successfully replicated previous research showing N400 responses to incongruent actions, and is the first to report an increase in mu power in response to observed incongruent actions compared to congruent actions. The timing of the mu power increase closely resembles those found in response to incongruent action words, suggesting that sensorimotor systems are involved in semantic action understanding across modalities. Such an involvement is supportive of the hypothesized roles of the MNS and Embodied Cognition in action understanding. We hypothesize that the interhemispheric interactions between N400 effect and mu power change likely reflect location specific functions of mu rhythm and N400 effects in action understanding.

Disclosures: **R.D. Soohoo:** None. **E. Van:** None. **J.A. Pineda:** None.

Nanosymposium

491. Perception and Imagery

Location: 147A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 491.10

Topic: A.10. Adolescent Development

Support: PA Dept of Health SAP grant 4100047862

Title: Increased developmental specialization from adolescence to adulthood in the face-processing network when viewing human faces versus animal faces

Authors: *E. WHYTE[1], S. SCHERF[2];
[2]Psychology, [1]Penn State Univ., University Park, PA

Abstract: There is developmental change in the core regions of the face-processing neural network, including the fusiform face area (FFA), in the transition from adolescence to adulthood. For example, the FFA increases in size across development (Scherf et al., 2007), which is related to improved face recognition behavior (Golarai et al., 2010). This may reflect increasing visuoperceptual expertise for recognizing human faces (Scherf et al., 2007). We evaluated the specificity of increasing specialization for face processing by contrasting age-related changes in neural sensitivity to human and animal (cat and dog) faces. Animal faces have similar featural and configural properties as do human faces and evoke significant (though reduced) FFA activation in adults (Halgren et al., 2000). We hypothesized that if increasing expertise explains the increasing specialization for face processing, we could observe developmental changes only in response to human, but not animal, faces. Using fMRI, we tested 13 adolescents (13 to 17 years) and 13 adults (18 to 24), matched on gender and handedness, in a 1-back memory task while viewing pictures of human faces, animal (cat and dog) faces, and common objects. Outside the scanner, participants completed the Cambridge Face Memory Task (CFMT; Duchaine and Nakayama, 2006). We defined individual face-processing regions using separate contrasts for human (vs objects) and animal (vs objects) faces. Adults exhibited larger human-defined FFA ROIs bilaterally than adolescents ($p < .05$). The groups did not differ in the size of the animal-defined FFA, or in the magnitude of activation for the FFA ROIs. Performance on the CFMT predicted increased human-defined FFA size across participants. A whole-brain voxelwise group comparison of human face activation revealed that both core (bilateral FFA) and extended regions (left anterior temporal pole, posterior cingulate gyrus, and dorsal medial PFC) show greater activation in adults compared to adolescents. This result stands in contrast to recent work reporting hyperactivation of the extended regions in children during a passive viewing task (Haist et al., 2013). In response to animal faces, adults exhibited increased activation only in the dorsal medial PFC. No regions showed greater activation in the adolescents. These results suggest that there is increasing activation in both the core and extended face-processing network during the transition from adolescence to adulthood for human, but not animal, faces. This specialization for human faces is likely due to increasing behavioral expertise for human faces.

Disclosures: E. Whyte: None. **S. Scherf:** None.

Nanosymposium

491. Perception and Imagery

Location: 147A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 491.11

Topic: A.10. Adolescent Development

Support: NIMH grant R01MH091864

NSF DGE-1144087

Title: Hebbian-like mechanism for human amygdala-mPFC network development

Authors: *L. GABARD-DURNAM[1], D. G. GEE[2], B. GOFF[2], J. FLANNERY[2], E. H. TELZER[3], K. L. HUMPHREYS[2], D. LUMIAN[2], D. S. FARERI[1], C. J. CALDERA[2], N. TOTTENHAM[1];
[1]Columbia Univ., New York, NY; [2]UCLA, Los Angeles, CA; [3]Univ. of Illinois at Urbana-Champaign, Urbana-Champaign, IL

Abstract: The mechanisms for establishing resting-state (RS) networks during development and how this RS functional connectivity (FC) relates to environmentally-induced task FC are unclear. Recent evidence suggests that experience-driven task activity may influence RS FC, a mechanism that is highly relevant during development when neural systems are most plastic to environmental inputs. This study directly compared the developmental patterns of RS and task-modulated FC across a 2-year period for an amygdala-medial prefrontal cortex (mPFC) network from early childhood through young adulthood (n =54, ages 4-23 years). Cross-sectionally, we found that the emotion task and RS FC for this amygdala-mPFC network had different age-related maturation patterns ($p < 0.05$, corrected), consistent with previous reports. We identified a highly negative relation between emotion task FC and RS FC that was not uniform across development, such that negative task FC was associated with positive RS FC more strongly in youths older than 10 years (n =38, p =0.027). Mediation analysis revealed that RS FC maturation was fully driven by changes in the nature of task FC. A longitudinal subsample further showed a unidirectional, developmentally causal influence of task FC on RS FC, where the nature of an individual's task FC predicted the nature of RS FC measured two years later (n =26, p =0.038), but RS FC could not predict future task FC. Together these findings suggest a neural-systems level Hebbian-like mechanism of functional connectivity development, whereby environmentally-induced phasic connectivity helps shape the nature of tonic RS connectivity measures.

Disclosures: L. Gabard-Durnam: None. **D.G. Gee:** None. **B. Goff:** None. **J. Flannery:** None. **E.H. Telzer:** None. **K.L. Humphreys:** None. **D. Lumian:** None. **D.S. Fareri:** None. **C.J. Caldera:** None. **N. Tottenham:** None.

Nanosymposium

491. Perception and Imagery

Location: 147A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 491.12

Topic: A.10. Adolescent Development

Support: NIH MH067924

Title: Protracted development of brain systems underlying working memory into early adulthood: A longitudinal fMRI study

Authors: *D. SIMMONDS[1], B. LUNA[2];
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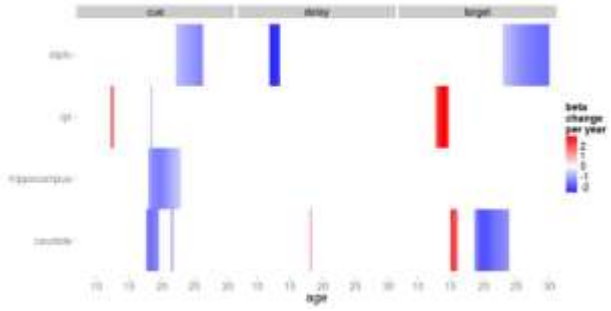
Abstract: Working Memory (WM), the ability to retain and manipulate information on-line to guide goal directed behavior, shows protracted development through adolescence and into young adulthood [Luna et al., 2004]. Cross-sectional studies using functional magnetic resonance imaging (fMRI) have found age related increases and decreases in the magnitude of activity regions supporting WM including dorsolateral prefrontal cortex (DLPFC) and posterior parietal cortex (PPC). These discrepancies could be due in part to cohort effects inherent in cross-sectional designs or to different stages of working memory processing. In this longitudinal study, 143 neurotypical individuals ages 8-30 performed a memory-guided saccade (MGS) task during fMRI. Participants were scanned annually for up to 8 years for a total of 378 scans, and mixed models combined with bootstrapping were used to assess rates of change in WM-related activity across development, and identify significant times of growth and maturation. The MGS task was designed to infer brain activation during WM stages of encoding, maintenance, and retrieval. Results showed decreases in MGS latency ($p=9.8 \times 10\text{-}14$) and improved accuracy ($p=0.01$) with age. In the brain, regional development varied by task stage. For encoding, activity decreased in late adolescence/early adulthood in the DLPFC ($p=0.03$), hippocampus ($p=0.0009$) and striatum ($p=0.008$) in a hierarchical manner, such that striatum matured earliest (17.7-19.5, 21.4-21.8), hippocampus matured next (18-22.9) and DLPFC matured last (22.2-26.2). There were no significant developmental changes during maintenance. During retrieval, there were significant changes in striatal activity ($p=0.006$), with mid-adolescence increases in activity (15-16) and early adulthood decreases (18.7-23.8). These results suggest primarily developmental decreases in engagement of WM circuitry in the context of improved WM performance that are specific to

different stages of WM processing and stages of development.



Disclosures: **D. Simmonds:** None. **B. Luna:** None.

Nanosymposium

491. Perception and Imagery

Location: 147A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 491.13

Topic: A.10. Adolescent Development

Support: NIH grant R01-MH59785

NIH grant P50-MH084051

NIH grant P30-HD003352

NIH Grant T32 MH018931

NIH Grant P50 MH100031

Title: Intrapair difference in childhood cortisol predict coping styles and affective brain function in adolescence

Authors: *C. A. BURGHY[1,2], M. E. FOX[3], D. CORNEJO[4], D. E. STODOLA[3], C. A. VAN HULLE[3], P. OJIAKU[3], R. M. BIRN[4], H. GOLDSMITH[5], R. J. DAVIDSON[6];
[1]Waisman Ctr. / Psychology, Waisman Ctr., Madison, WI; [3]Waisman Ctr., [4]Psychiatry, [5]Psychology / Waisman Ctr., [6]Psychology / Psychiatry / Waisman Ctr., [2]Univ. of Wisconsin-Madison, Madison, WI

Abstract: Affect dysregulation in adolescence is common and attributed in part to the continued development of the prefrontal cortex (PFC), particularly its reciprocal relations with the

amygdala and hypothalamic-pituitary-adrenal axis. Recent work has demonstrated potentially cascading deleterious effects stemming from early life stress exposure and atypical basal cortisol function in childhood to aberrant resting-state functional connectivity (rs-FC) and psychiatric symptoms in adolescence. It remains unclear whether these differences were truly the result of early experience and/or due to genetic factors. We investigate these relations using a prospective monozygotic (MZ) twin design. Specifically, we examine whether intrapair differences in late afternoon cortisol function at age 7 predict intrapair differences in brain activity in adolescence in genotypically-identical MZ twins. Method: Rs-FC and emotion regulation fMRI task data were collected from 20 adolescent MZ twin pairs (10 female pairs; Mage = 15.5 yrs) selected from a longitudinal twin study. In the task, participants viewed emotionally-valenced images; for a portion of the trials a picture of a neutral male face was presented briefly following picture offset. These data were then examined in relation to childhood afternoon basal cortisol levels and adolescent self-reported coping strategies. Results: Using an amygdala-seeded voxel-wise approach, we found that intrapair differences in childhood cortisol prospectively predicted intrapair differences in amygdala-pgPFC rs-FC ($R2 = 0.84$, FWE-corrected $p = .01$), such that the twin with higher cortisol in childhood evinced lower connectivity in this crucial pathway in adolescence. Higher intrapair differences in childhood cortisol also predicted differences in amygdala recovery following unpleasant images ($t = 4.34$, SV-corrected $p < .05$). Here, twins with relatively higher cortisol had more prolonged amygdala activity suggesting poorer affect regulation. Last, co-twin differences in amygdala recovery predicted coping styles, where co-twins with relatively poorer amygdala recovery were more likely to endorse less effective coping strategies and vice versa. Conclusions: MZ intrapair differences in childhood late afternoon cortisol levels predict intrapair differences in affect-related measures of brain function. These differences also predict coping styles. By using the MZ Difference Design, we highlight associations that share non-genetic underpinnings. Thus, these longitudinal results illuminate a nexus of hormonal, behavioral, and neural measures of affect regulation that is experience-dependent.

Disclosures: **C.A. Burghy:** None. **M.E. Fox:** None. **D. Cornejo:** None. **D.E. Stodola:** None. **C.A. Van Hulle:** None. **R.M. Birn:** None. **H. Goldsmith:** None. **R.J. Davidson:** None. **P. Ojiaku:** None.

Nanosymposium

491. Perception and Imagery

Location: 147A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:30 AM

Presentation Number: 491.14

Topic: F.01. Human Cognition and Behavior

Support: Swiss National Science Foundation Grant K-13K1-119953 to Katharina Henke

Title: Learning words during sleep

Authors: *S. RUCH[1,2], T. KOENIG[3,2], J. MATHIS[4], C. ROTH[4], K. HENKE[1,2];
[1]Univ. of Bern, Dept. of Psychology, Bern, Switzerland; [2]Univ. of Bern, Ctr. for Cognition, Learning and Memory, Bern, Switzerland; [3]Univ. of Bern, Univ. Hosp. of Psychiatry, Dept. of Psychiatric Neurophysiol., Bern, Switzerland; [4]Univ. of Bern, Inselspital, Univ. Hospital, Dept. of Neurol., Bern, Switzerland

Abstract: Humans process and understand spoken language during sleep. This is indicated by studies which recorded brain responses that were induced if sleeping subjects listened to congruent versus incongruent sentences or heard their own name versus names of others. If we understand language, we might also be able to learn new verbal information while sleeping. But evidence for this claim is missing. However, a recent study indicated that humans can learn to take deeper breaths when hearing a tone that was repeatedly associated with a pleasant odor during sleep. Learning during sleep is thus possible and should be feasible with verbal information too. To investigate verbal learning during sleep, we played words to participants while they were taking a nap. Word presentation was confined to non-rapid eye movement (NREM) sleep because this sleep stage is vitally involved in memory consolidation. We tested participant's memory for sleep-played words following waking. Word retrieval was assessed with two implicit (indirect) tests that measure the effect of memory on behavior in the absence of conscious recollection of the learning event. The first test assessed whether playing words during sleep facilitates the identification of semantically related words after waking up (semantic priming). The second test measured whether participants respond faster when choosing the sleep-played word over a foil word in a forced-choice task (perceptual priming). Participants processed words during sleep as was indicated by the word-evoked up-states in the encephalogram. Up-states are NREM sleep-specific phases of briefly increased neuronal excitability that might promote learning during sleep. Participants who responded to words with particularly large up-states stored words for use following waking. The greater the evoked up-states, the stronger the semantic and perceptual priming effects in the two retrieval tasks administered following waking. We conclude that humans understand verbal messages during sleep and can store them for later use. Sleep-played messages may thus affect behavior following sleep.

Disclosures: S. Ruch: None. **T. Koenig:** None. **J. Mathis:** None. **C. Roth:** None. **K. Henke:** None.

Nanosymposium

492. Human Reinforcement Learning: Development and Aging

Location: 150A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 492.01

Topic: F.01. Human Cognition and Behavior

Support: NSF GFRP (DGE-1137475) to MRC

NIH-MH80603 to GAB and RMS

NIH-MH091451 to RMS

Title: Paradoxical neurobehavioral rescue by cues associated with infant trauma: Amygdala serotonin (5-HT) and corticosterone (CORT)

Authors: *M. RINCÓN CORTÉS[1,2,3], G. A. BARR[4,5], A. M. MOULY[6], K. SHIONOYA[7], S. NUNEZ[8], R. M. SULLIVAN[1,2,3];
[1]Neurosci. and Physiol., NYU Sackler Inst. at the NYU Sch. of Med., New York, NY; [2]Emotional Brain Inst. at the Nathan Kline Inst., Orangeburg, NY; [3]Child and Adolescent Psychiatry, Child Study Ctr. at the NYU Sch. of Med., New York, NY; [4]Anesthesia and Critical Care Med., Children's Hosp. of Philadelphia, Philadelphia, PA; [5]Perelman Sch. of Med., Philadelphia, PA; [6]Lyon Neurosci. Res. Center, INSERM U1028, CNRS UMR5292, Univ. Lyon1, Lyon, France; [7]Div. of Cell Biology, Dept. of Clin. and Exptl. Med., Linköping Univ. IKE, Linköping, Sweden; [8]Dominican Col., Orangeburg, NY

Abstract: Early life trauma, especially within the context of the caregiver, is associated with compromised socioemotional development, increased susceptibility for depression, amygdala dysfunction and serotonin (5-HT) alterations (Rincón-Cortés and Sullivan, 2014). We explore the complex interaction between infant trauma's immediate and long-term effects. The immediate effect is that the infant learns an attraction to cues associated with the trauma, although trauma also produces depressive-like behavior that emerges later in development. Paradoxically, one might expect that cues associated with infant trauma are avoided, yet we show data suggesting that these cues are powerfully attractive to the infant and can rescue depressive-like behavior when presented in adulthood. Our previous work has shown that odors associated with trauma (i.e., shock) engage the infant learning system, which is predisposed for attachment preference learning instead of the typical adult aversion learning (Sullivan et al., 2000). This infant learning system is robust and ensures the infant learns the maternal odor and forms an attachment to the caregiver regardless of quality of care. However, there are long-term consequences of infant trauma, including depressive-like behavior and amygdala dysfunction. Recently, we found that the learned maternal odor retains its powerful neurobehavioral effects

into adulthood - it rescues depressive-like behavior (Sevelinges et al., 2011). We used an artificial maternal odor-learning paradigm during the sensitive period for attachment learning in rat pups (postnatal days (PN) 8-12) and assessed the mechanism by which this artificial maternal odor is able to rescue adult depressive-like behavior. Infant trauma, as modeled by odor-shock conditioning, causes an atypical amygdala 5-HT increase. Moreover, increasing pups' 5-HT (i.e., fluoxetine) is sufficient to produce later-life depressive-like behavior, as shown through testing animals on the Forced Swim Test (FST). Importantly, the presence of this odor in adulthood rescues depressive-like behavior, aberrant amygdala paired-pulse inhibition and amygdala gene expression related to glucocorticoid/5-HT signaling. A causal role of glucocorticoid/5-HT in the later-life rescue was demonstrated by blocking amygdala 5-HT, which prevented the odor-mediated rescue, whereas increasing amygdala 5-HT and blocking CORT mimicked the odor rescue effect in the FST. Taken together, these results suggest that artificial maternal odor has properties reminiscent of safety signals, which can be acquired in infancy and rescue adult depressive-like behavior by modulating amygdala 5-HT.

Disclosures: **M. Rincón Cortés:** None. **G.A. Barr:** None. **A.M. Mouly:** None. **K. Shionoya:** None. **S. Nunez:** None. **R.M. Sullivan:** None.

Nanosymposium

492. Human Reinforcement Learning: Development and Aging

Location: 150A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 492.02

Topic: F.01. Human Cognition and Behavior

Support: NSF GRFP DGE-1058262

NIH R01 MH099078

Title: The development of hierarchical rule learning and generalization in infancy

Authors: ***D. WERCHAN**, M. J. FRANK, D. AMSO;
Brown Univ., Providence, RI

Abstract: Introduction: The ability to extract and generalize abstract rules from experience is a hallmark of cognition that helps guide learning in new environments. Behavioral evidence suggests that adults will spontaneously impute structure into simple learning problems, which affords potential generalization opportunities in novel environments (Collins and Frank, 2013).

This process of abstract or hierarchical rule learning is supported by interactions between the prefrontal cortex and striatum via dopaminergic pathways (Collins and Frank, 2013; Collins, Cavanagh, and Frank, 2014). Yet, little is known about how these processes occur in infants. Here we examined whether young infants could infer hierarchical rules and generalize learned information to novel contexts. **Methods:** Twenty 7-9 month infants (M = 8.5, SD = 1.22) participated in a learning phase and a generalization test phase. During each phase, infants were presented with stimulus-reward location pairs. The stimuli varied by shape and color and the reward-location consisted of a cartoon animation presented in specific location of the screen. These pairs were constructed such that an implicit hierarchical order could be inferred, with some features acting as higher-order contexts indicative of a task set and other features acting as lower-order stimuli indicative of the reward location given the task set. Infants' saccade latencies to the reward locations were measured. If the infants were learning the associations, we expected to see a decrease in saccade latencies over trials. We also measured blink rate—a known proxy for central dopamine activity (Karson, 1982)— during learning. **Results:** We found that infants inferred the hierarchical structure and abstracted two task sets from the input. Moreover, infants were able to generalize one of these task sets to support learning in a novel context. Blink rate during learning of the task sets also predicted generalization at test, suggesting that this ability may be supported by central dopamine activity. These findings demonstrate that infants may infer abstract rule representations during learning, affording generalization in new contexts. This research provides novel insight into the early emergence of hierarchical rule abstraction and learning in infancy.

Disclosures: D. Werchan: None. **M.J. Frank:** None. **D. Amso:** None.

Nanosymposium

492. Human Reinforcement Learning: Development and Aging

Location: 150A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 492.03

Topic: F.01. Human Cognition and Behavior

Support: NIH grant DA 035050

NIH grant MH 048404-23

Title: Adolescent ventral tegmental area neurons maintain cue-evoked responding after extinction: A mechanism for adolescent behavioral flexibility?

Authors: *N. W. SIMON[1], J. WOOD[2], Y. KIM[2], B. MOGHADDAM[2];
[1]Neurosci., [2]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Dopamine neurons in the ventral tegmental area (VTA) are strongly implicated in adolescent behavioral and psychiatric vulnerabilities, but little is known about how adolescent VTA neurons process reward and other behaviorally relevant information. Here, we assessed neuronal activity in VTA during acquisition and extinction of instrumental behavior in adolescent and adult rats. Rats were initially trained to perform a nose-poke to receive a pellet reward, then were given a session of extinction in which the reward was never available. During learning, putative dopamine neurons developed similar phasic responses to a cue predicting reward availability in both age groups, but showed larger responses before and after reward delivery in adults compared to adolescents. Simultaneously recorded putative non-dopamine neurons were activated by instrumental actions in adolescents, but not adults. During extinction, adult putative dopamine neurons rapidly diminished their response to the cue, whereas adolescent neurons remained activated by the cue. This difference in extinction-related cue processing occurred despite both age groups similarly learning the task and quickly adapting behavior to extinction. To elucidate the behavioral consequences of this perseverative cue-evoked neuronal responding, we ran separate groups of adolescent rats in experiments testing cue- and reward-driven behavior. We found no difference between adolescent and adult rats in extinction or outcome-driven reinstatement, indicating that cues are likely not more tightly linked to outcomes in adolescents than adults. However, when a learned instrumental cue was shifted in modality to a Pavlovian cue, adolescent rats flexibly acquired an approach response (goal-tracking) to this cue more readily than adults. Finally, a cue initially acquired as a response inhibitor was shifted to a Pavlovian predictor of reward. Adolescent rats again learned to goal-track in response to the cue following the shift in value more rapidly than adults. Thus, adolescents are able to effectively adjust responding to changes in cue-outcome relationships compared to adults. Persistent cue-evoked responding by the adolescent reward system after extinction may facilitate the ability of previously salient cues to rapidly acquire changes in value.

Disclosures: **N.W. Simon:** None. **J. Wood:** None. **Y. Kim:** None. **B. Moghaddam:** None.

Nanosymposium

492. Human Reinforcement Learning: Development and Aging

Location: 150A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 492.04

Topic: F.01. Human Cognition and Behavior

Support: NIMH R01 MH091864

Title: Normative development of ventral striatal resting-state connectivity in humans

Authors: *D. S. FARERI, L. GABARD-DURNAM, B. GOFF, J. FLANNERY, D. G. GEE, D. S. LUMIAN, C. J. CALDERA, N. TOTTENHAM;
Psychology, UCLA, Los Angeles, CA

Abstract: Incentives play a crucial role in guiding behavior throughout our lives, but perhaps no more so than during the early years of life. It is well established that the ventral striatum is a critical piece of an incentive-based learning circuit, sharing afferent and efferent anatomical connections with subcortical (e.g., amygdala, hippocampus) and cortical structures (e.g., medial prefrontal cortex; mPFC) additionally supporting incentive valuation and learning (Haber and Knutson, 2010). Resting-state functional connectivity (rsFC) is a powerful and non-invasive method by which to track the development of functional relationships between neural structures involved in incentive-based learning. rsFC may reflect maintenance of relationships between regions involved in related cognitive processes (Vincent and Buckner, 2007). We employed a seed-based correlation approach to investigate ventral striatal rsFC in a cross-sectional sample of typically developing individuals between the ages of 4 and 23 years old (n=66). We specifically probed changes in ventral striatal rsFC with targeted a priori regions implicated in incentive-based processes—the amygdala, hippocampus, mPFC (ACC, vmPFC, OFC), and insula. Ventral striatum was strongly positively connected to amygdala and mPFC in childhood; specific mPFC regions (subgenual anterior cingulate cortex; sgACC) demonstrated significant age-related declines in positive connectivity, while others (vmPFC, OFC) remained strongly positively coupled. Connectivity between the ventral striatum and hippocampus emerged in adulthood, consistent with task-based studies showing hippocampal recruitment in incentive-based processes in adults (Foerde and Shohamy, 2011, Barron et al., 2013). Ventral striatal-insula connectivity exhibited significant quadratic changes across age, characterized by decreases in connectivity during adolescence. Finally, given associations between testosterone and maturation of ventral striatal function in adolescence (Op de Macks et al., 2011) we examined changes in testosterone levels as a mechanism underlying ventral striatal rsFC development. Decreased rsFC between ventral striatum and sgACC was associated with increased levels of testosterone. In sum, our findings suggest normative ventral striatal rsFC development is dynamic and characterized by early establishment of connectivity with medial prefrontal and limbic structures supporting incentive-based learning, as well as substantial functional reorganization with later developing regions during transitions into and out of adolescence.

Disclosures: D.S. Fareri: None. **L. Gabard-Durnam:** None. **B. Goff:** None. **J. Flannery:** None. **D.G. Gee:** None. **D.S. Lumian:** None. **C.J. Caldera:** None. **N. Tottenham:** None.

Nanosymposium

492. Human Reinforcement Learning: Development and Aging

Location: 150A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 492.05

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant DA034316

Title: Changes in sensitivity to value magnitude from childhood to adulthood relate to risk-taking behavior

Authors: *S. M. HELFINSTEIN[1], J. A. MUMFORD[2], M. E. DUNN[3], J. R. ANTHIS[3], J. L. LEAKE[4], K. FROMME[2], R. A. POLDRACK[1];
[1]Imaging Res. Ctr., [2]Dept. of Psychology, [3]The Univ. of Texas At Austin, Austin, TX; [4]The Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX

Abstract: Risk-taking behavior increases substantially from childhood to the adolescent and young adult years. Previous research has suggested that this phenomenon is partially driven by a peak in neural prediction error response to reward during adolescence. However, naturalistic public health relevant risks often have a particular high-variance feedback structure, with frequent small rewards coupled with rare—but devastatingly severe—negative outcomes. In light of this pattern, we examined how the ability to learn to evaluate risks with high-variance and low-variance outcome structures changes between the ages of 8 and 30. While undergoing an fMRI scan, subjects performed a learning task in which they made choices about four different "point machines," which varied on expected value (positive or negative) and outcome variance (high or low). Subject choice data was fitted to a modified prediction error model with an additional parameter reflecting the concavity of the value function. This concavity factor correlated significantly with age, with younger subjects showing a greater tendency to underweight large values, accounting for their poor task performance in high-variance outcome structures. The concavity of the value function also correlated with subject performance on the Balloon Analog Risk Task—a laboratory measure known to reflect public health relevant risk-taking—even after accounting for age effects. These findings suggest that differences in sensitivity to value magnitude may partially explain the increase in risk-taking behavior seen as children move into adolescence. Prediction error responses to trial outcomes derived from each individual's model parameters were used as regressors on the imaging data. A prediction error pattern of response to positive feedback was seen in the striatum and medial frontal cortex, while prediction error response to negative feedback was seen in the anterior insula. These results suggest that the changes in sensitivity to value magnitude seen in the behavioral data may be related to developmental changes in the scaling of neural prediction error responses.

Disclosures: **S.M. Helfinstein:** None. **J.A. Mumford:** None. **M.E. Dunn:** None. **J.R. Anthis:** None. **J.L. Leake:** None. **K. Fromme:** None. **R.A. Poldrack:** None.

Nanosymposium

492. Human Reinforcement Learning: Development and Aging

Location: 150A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 492.06

Topic: F.01. Human Cognition and Behavior

Support: NSF-GRFP DGE-11-44155

NSF Early Career Award 0955454

NINDS R01-078784

NSF BCS-0963750

Title: Multiple learning systems in adolescence

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Abstract: Adolescence is a stage of development characterized by many behavioral and neural changes. There have been many recent advances in understanding the neural mechanisms of risky decision-making and reward sensitivity in adolescence. However, much less is known about how the adolescent brain learns to predict rewards and whether reward sensitivity in adolescence modulates other forms of learning. Here we aimed to address this gap. Guided by findings of hyper-activation of the striatum related to heightened feedback sensitivity, we hypothesized that in a reinforcement learning paradigm adolescents will perform better than adults when learning incrementally from outcomes. Furthermore, we hypothesized that increased reward sensitivity in adolescence could have consequence for other learning systems, such as the hippocampus, known for binding across stimulus-stimulus associations. To test these hypotheses, we investigated feedback learning in healthy adolescents under simple reinforcements as they underwent event-related fMRI. We employed a probabilistic feedback learning task where outcomes on each trial were followed by "correct" and "incorrect" feedback. We compared healthy adolescents (13-17 years old) and healthy adults (24-30 years old). Behaviorally, we found that adolescents showed better learning than adults ($Z=2.56$, $p<0.01$). To investigate the

underlying learning mechanisms, we fit a reinforcement-learning model to examine trial-by-trial learning related responses in both age groups. The learning rate among adolescents was lower than in adults ($t(46)=2.11$, $p<0.05$), suggesting that their learning was more incremental. Using the reinforcement learning model to estimate prediction errors for each subject, we found that adolescents displayed heightened prediction error related activity in the striatum (p-FWE<0.05). Moreover, adolescents displayed greater prediction error activity in the hippocampus (p-FWE<0.05), accompanied by enhanced episodic memory for feedback events ($F(1,95)=5.22$, $p=0.03$). Taken together our findings suggest that heightened sensitivity to feedback in adolescents confers a benefit for striatal dependent learning, and that heightened activity in the striatum may have downstream behavioral consequences for other types of learning and memory.

Disclosures: **J.Y. Davidow:** None. **K. Foerde:** None. **A. Galván:** None. **D. Shohamy:** None.

Nanosymposium

492. Human Reinforcement Learning: Development and Aging

Location: 150A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 492.07

Topic: F.01. Human Cognition and Behavior

Title: Neural reactions to positive and negative feedback change across adolescent development

Authors: ***S. PETERS**[1], B. R. BRAAMS[1], A. C. K. VAN DUIJVENVOORDE[1], P. C. M. P. KOOLSCHIJN[2], E. A. CRONE[1];
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Abstract: The ability to learn from feedback is an important contributor to successful performance in school. Despite the progress in unraveling the neural correlates of cognitive control in childhood and adolescence, relatively little is known about how these brain regions contribute to feedback learning. In this longitudinal study, 268 participants aged 8-25 years performed a feedback-learning task in a 3T MRI scanner. We examined the development of neural reactions to positive and negative feedback, and its relevance for school-based measures such as reading. The prefrontal cortex showed more activation following negative compared to positive feedback with increasing age. In addition, the parietal cortex demonstrated a shift from sensitivity to positive feedback in young children to negative feedback in adolescents and adults. The longitudinal data demonstrate reliable patterns of activation within individuals, which correlate with behavioral performance.

Disclosures: **S. Peters:** None. **B.R. Braams:** None. **A.C.K. Van Duijvenvoorde:** None. **P.C.M.P. Koolschijn:** None. **E.A. Crone:** None.

Nanosymposium

492. Human Reinforcement Learning: Development and Aging

Location: 150A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 492.08

Topic: F.01. Human Cognition and Behavior

Title: Experiential learning outweighs instruction early in development

Authors: *J. H. DECKER[1,2], F. S. LOURENCO[1,2], B. B. DOLL[3], B. CASEY[1,2], C. A. HARTLEY[1,2];
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Abstract: Throughout our lives, we face the important task of distinguishing rewarding actions from those that are best avoided. Importantly, there are multiple means by which we acquire this information. Through trial and error, we use experiential feedback to evaluate our actions. We also learn which actions are advantageous through explicit instruction from others. Here, we examined whether the efficacy of these two forms of learning changes across development by placing instruction and experience in competition in a probabilistic learning task, adapted for use across development. 31 children (ages 6-12), 31 adolescents (ages 13-17), and 26 adults (ages 18-34) learned through trial and error to select the most rewarded option for each of three pairs of stimuli. One lower-valued stimulus was falsely instructed as being a good choice. After this learning phase, participants chose between all 15 possible stimulus pairings without feedback, enabling evaluation of the degree to which participants' learned the true value of each stimulus, or whether instruction biased this learning. Preliminary data suggest that whereas inaccurate instruction markedly biased adults' value estimation, children and adolescents relied chiefly on their experience. Instructional control of learning is thought to recruit corticostriatal brain circuitry, which continues to mature into adulthood. These behavioral data suggest that this protracted neurocognitive maturation may bias children and adolescents to learn less effectively from explicit instructions than they do their own previous experience.

Disclosures: **J.H. Decker:** None. **F.S. Lourenco:** None. **C.A. Hartley:** None. **B. Casey:** None. **B.B. Doll:** None.

Nanosymposium

492. Human Reinforcement Learning: Development and Aging

Location: 150A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 492.09

Topic: F.01. Human Cognition and Behavior

Support: Mortimer D. Sackler, M.D. family

Title: The developmental emergence of model-based learning

Authors: *C. A. HARTLEY[1], J. H. DECKER[1], A. R. OTTO[2], N. D. DAW[2], B. CASEY[1];
[1]Sackler Inst. For Developmental Psychobiology, Weill Cornell Med. Col., NEW YORK, NY;
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Abstract: Psychological theories distinguish "goal-directed" actions, performed to obtain desired future outcomes, from "habits", actions rendered stimulus-bound and automatic through previous reinforcement. Goal-directed action is proposed to rely upon "model-based" learning, which recruits a cognitive model of the consequences of potential actions, enabling flexible adaptation to a dynamic environment. In contrast, habits are thought to recruit a more efficient "model-free" learning process that attaches an action value to a stimulus, allowing well-honed behavioral routines to be executed without forethought or attention. Model-based learning is proposed to recruit prefrontal-subcortical circuitry, which undergoes substantial structural and functional changes during maturation from childhood into adulthood. While this suggests that individual reliance upon model-based versus model-free learning might change markedly with age, the developmental trajectory of action selection strategies has not yet been examined. In this study, children, adolescents, and adults performed a two-stage reinforcement-learning task in which we can estimate model-based and model-free contributions to choice behavior. Our data suggest that while the behavioral signature of model-free learning is present from childhood onwards, model-based choice is not evident until adolescence, and continues to mature into adulthood. We present a provisional model of the neural changes underlying this developmental emergence of model-based learning, and suggest that this protracted cognitive maturation may contribute to the shortsighted decision-making that is commonly observed during adolescence.

Disclosures: C.A. Hartley: None. **J.H. Decker:** None. **B. Casey:** None. **N.D. Daw:** None. **A.R. Otto:** None.

Nanosymposium

492. Human Reinforcement Learning: Development and Aging

Location: 150A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 492.10

Topic: F.01. Human Cognition and Behavior

Support: German Federal Ministry of Education and Research (BMBF) Grant FKZ 01GQ0913

German Federal Ministry of Education and Research (BMBF) Grant FKZ 01GQ1313

Title: Prefrontal deficits in older adults implicate a shift from model-based to model-free learning and decision-making strategies

Authors: *B. EPPINGER[1], H. R. HEEKEREN[2], S.-C. LI[1,3];
[1]TU Dresden, Dresden, Germany; [2]Freie Univ. Berlin, Berlin, Germany; [3]Max Planck Inst. for Human Develop., Berlin, Germany

Abstract: Strategic decision-making depends on the ability to learn the value of future rewards and the sequential decisions that are necessary to achieve them. In two recent studies we investigated age-related changes in the learning and application of sequential task structures that lead to future reward. In the first study we used a three-state Markov decision task and functional MRI to investigate age differences in the neural systems that mediate the learning of sequential transition structures. The results show that in younger adults learning was associated with enhanced activity in the prefrontal cortex (PFC). In older adults we found no evidence for PFC recruitment. However, high-performing older adults showed enhanced activity in the ventral striatum, suggesting that they might engage in a model-free learning strategy. Using change point analyses we show that in younger adults learning was characterized by distinct and abrupt shifts in PFC activity, which were predictive of transitions in choice behavior. In older adults PFC activity was delayed, less pronounced and not predictive of behavior. Our findings suggest that age-related impairments in learning to predict future reward can be attributed to prefrontal deficits during the extraction of sequential state transition structures. In the second study we examined the interplay of model-based and model-free decision mechanisms in younger and older adults using a two-stage Markov decision task. The results of this study show a shift from model-based to model-free decision-making with age. This shift in decision strategies is particularly pronounced in situations in which unexpected rewards signal the need for behavioral adaptations. In these situations, younger adults use their knowledge of the task structure to optimize decision-making, whereas older adults show perseverative behavior. Taken together, our results show that age-related impairments in the learning and application of model-based

representations are related to functional deficits in the PFC. Under high load on strategic learning and decision-making processes older adults seem to converge on a model-free decision-making strategy that relies on striatal learning mechanisms. We think that our results have a broader societal relevance because many of our everyday decision problems involve learning about underlying (partially observable) structures and may only be insufficiently solved using a model-free strategy. That is, deficits in the ability to learn sequential state structures (such as in older adults) may lead to myopic and inflexible decision-making.

Disclosures: B. Eppinger: None. **H.R. Heekeren:** None. **S. Li:** None.

Nanosymposium

492. Human Reinforcement Learning: Development and Aging

Location: 150A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 492.11

Topic: F.01. Human Cognition and Behavior

Support: NIA grant AG043425

NIDA grant DA032457

Title: State-based versus reward-based decision-making in younger and older adults

Authors: *D. WORTHY[1], W. MADDOX[2];
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Abstract: Normal aging is associated with a number of declines in cognitive processing. Even so, older adults show deficits in some forms of decision making but advantages in other forms. We hypothesize that normal aging is associated with a shift away from model-based processing (associated with optimal state-based decision making) and toward model-free processing (associated with optimal reward-based decision-making). We also hypothesize that older adults are more efficient than younger adults at selecting strategies that optimize performance in the face of reduced cognitive resources. We test these hypotheses in older and younger adult state-based and reward-based decision-making using behavioral testing and computational modeling. We find that an age-related advantage in state-based and reward-based decision-making when the number of decision options is small. Computational modeling suggests that this advantage is due to older adults reliance on a simple, but effective heuristic. We find that an age-related deficit in state-based decision-making and an age-related advantage in reward-based decision-

making when the number of decision options is large. Computational modeling suggests that this pattern of deficits and advantages is due to older adults increased reliance on model-free processing.

Disclosures: D. Worthy: None. **W. Maddox:** None.

Nanosymposium

492. Human Reinforcement Learning: Development and Aging

Location: 150A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 492.12

Topic: F.01. Human Cognition and Behavior

Title: Dopamine contributions to model-free and model-based learning

Authors: *M. SHARP[1], K. FOERDE[2], N. D. DAW[2], D. SHOHAMY[1];
[1]Psychology, Columbia Univ., New York, NY; [2]Psychology, New York Univ., New York, NY

Abstract: Reinforcement learning is thought to involve two distinct sorts of processes. The first, *model-free* learning, is exquisitely sensitive to reward, depends on immediate feedback and is computationally efficient. Its implementation follows temporal difference learning algorithms, and as such, model-free learning is postulated to depend critically on striatal dopamine signalling of prediction errors. The second, *model-based* learning, is also sensitive to feedback but additionally depends on working memory to maintain a complex internal representation of future states and on effective cognitive control to guide decisions. While model-based reinforcement learning appears to rely on a broader neural network, its exact neural representation remains incompletely mapped out. Furthermore, it remains unknown if dopamine is involved; either directly, by implementing model-based learning through nigro-striatal dopaminergic projections, or indirectly, through prefrontal dopamine contributions to working memory and cognitive control. Here, we aimed to address these questions by examining learning in patients with early stage Parkinson's disease, which is predominantly characterized by striatal dopamine loss. We tested the role of dopamine in modulating both model-free and model-based learning by assessing the performance of 21 patients with Parkinson's disease on a two-step sequential decision-making task previously shown to dissociate between these two learning processes. Patients were tested in two separate sessions, both ON and OFF dopaminergic medications, and performance was compared to 21 healthy age-matched controls and 10 young adults. We hypothesized that (i) PD patients OFF medications would show reduced model-free learning, that (ii) this deficit would be largely restored by dopamine replacement, and that (iii) healthy older

adults would show reduced model-based learning compared to young adults, guided by recent findings indicating a role for the prefrontal cortex. Contrary to predictions, we found that PD-OFF relied significantly more on model-free than model-based learning. Moreover, we found that dopamine replacement restored the normal balance between model-free and model-based learning in the PD-ON, such that PD-ON were not significantly different from the healthy older adults or young adults. Overall, our results provide evidence for dopaminergic control of model-based learning and suggest that model-based learning is particularly sensitive to the dopamine loss of early to moderate stage Parkinson's disease.

Disclosures: **M. Sharp:** None. **K. Foerde:** None. **N.D. Daw:** None. **D. Shohamy:** None.

Nanosymposium

492. Human Reinforcement Learning: Development and Aging

Location: 150A

Time: Tuesday, November 18, 2014, 8:00 AM - 11:15 AM

Presentation Number: 492.13

Topic: F.01. Human Cognition and Behavior

Support: BMBF-01GQ0914

BMBF-01GQ1001B

BMBF-01GQ0911

Title: Age-dependent interaction of novelty-driven exploration and reinforcement learning

Authors: ***A. HOUILLON**[1,2], R. LORENZ[3,5], T. GLEICH[3], A. HEINZ[4], J. GALLINAT[3], K. OBERMAYER[1,2];
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Abstract: We investigated how reward learning & its interaction with novelty-driven exploration could be affected by age. Stimulus novelty enhances exploratory choices through engagment of neural reward systems. SN/VTA activation by novelty in a rewarding context has raised the possibility that novelty per se might have intrinsic rewarding properties. However, SN/VTA activations to novelty alone also suggest a second, reward-independent mechanism, that favors directed exploration toward the novel cue. Therefore we proposed that novelty per se

- a form of unexpected uncertainty- can act as a directed explorative bias, but can also act as a bonus for rewards when these are explicitly attended. Interestingly, the noradrenergic system mediates learning from unexpected uncertainty & has also been shown to be linked to explorative behavior. It has further been suggested that the exploration-exploitation tradeoff could be mediated by the interaction of dopamine & noradrenaline systems, where dopamine is believed to be stronger associated with exploitative & noradrenaline with explorative behaviors. Older subjects tend to have lowered levels of dopamine & lower novelty-seeking scores. Therefore, we hypothesized that undirected explorative behavior should increase, but novelty-driven explorative behavior should decrease across the lifespan. We applied a reward-dependent learning task to young & older participants. Computational models were used to quantify differences in behavioral performance & fMRI activation. We showed that novel stimuli presented from a pre-familiarized category could accelerate or decelerate learning of the most rewarding category, depending on the individual sensitivity to novelty. Choices were quantified in reinforcement learning models, including parameters to characterize individual variation in novelty-driven exploration, & undirected exploration. As expected, the simulations showed that older subjects had lower novelty-driven explorative behavior, but undirected explorative behavior was increased. In addition, the age-induced increase of the undirected exploration parameter anticorrelated with working memory performance. fRMI analysis was performed by including predictions of the computational model. Striatum & midbrain where activated by the novelty contrast in trials with low probability of correct action, suggesting that novelty activates the reward system during the explorative learning phase. Cingulate cortex was activated by the contrast of familiar vs novel trials, suggesting that novelty also activates reward-independent exploration mechanism & may be related to the noradrenaline system.

Disclosures: **A. Houillon:** None. **K. Obermayer:** None. **R. Lorenz:** None. **T. Gleich:** None. **J. Gallinat:** None. **A. Heinz:** None.

Nanosymposium

576. Synapse Function in Development and Disease

Location: 147A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 576.01

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

Support: NIH grant MH095096

NIH grant GM089652

Title: High content image analysis identifies novel regulators of synaptogenesis in a high-throughput RNAi screen of primary neurons

Authors: *T. NIELAND[1], D. LOGAN[2], J. SAULNIER[3], D. LAM[2], C. JOHNSON[3], D. ROOT[2], A. CARPENTER[2], B. SABATINI[3];
[1]MIT, Cambridge, MA; [2]Broad Inst. MIT Harvard, CAMBRIDGE, MA; [3]Howard Hughes Med. Institute, Harvard Med. Sch., Boston, MA

Abstract: The formation of synapses, the specialized points of chemical communication between neurons, is a highly regulated developmental process fundamental to establishing normal brain circuitry. Perturbations of synapse formation and function causally contribute to human developmental and degenerative neuropsychiatric disorders, such as Alzheimer's disease, intellectual disability, and autism spectrum disorders. Many genes controlling synaptogenesis have been identified, but lack of facile experimental systems has made systematic discovery of regulators of synaptogenesis challenging. Thus, we created a high-throughput platform and automated synapse image analysis tools to study excitatory and inhibitory synapse development in primary neuronal cultures and used a lentiviral RNA interference library to identify novel regulators of synapse formation. This methodology is broadly applicable for high-throughput screening of genes and drugs that may rescue or improve synaptic dysfunction associated with cognitive function and neurological disorders.

Disclosures: T. Nieland: None. **D. Logan:** None. **J. Saulnier:** None. **D. Lam:** None. **C. Johnson:** None. **D. Root:** None. **A. Carpenter:** None. **B. Sabatini:** None.

Nanosymposium

576. Synapse Function in Development and Disease

Location: 147A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 576.02

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

Support: NIH grant MH096908

Title: Understanding mechanisms of synapse development through longitudinal *in vivo* imaging of identified presynaptic terminals before, during and after the critical period

Authors: T. EVANS[1], L. BURY[2], *S. L. SABO[3];
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Abstract: Understanding the dynamic mechanisms through which synapses are assembled and eliminated is essential to understanding cortical circuit development. Given the complexity of cortical circuitry and the protracted postnatal period of synapse development, during which synapses are formed and eliminated in parallel, the dynamics of synapse development are best addressed with longitudinal studies of synapses formed by the same neurons throughout circuit development. While many studies have used live imaging to study the dynamics of synapse development in vitro, few studies have examined the dynamics of postnatal neocortical synapse development in vivo, where sensory inputs are intact. In addition, previous studies of synapse formation and elimination have mainly focused on GFP or YFP filled dendritic spines, and most studies of presynaptic bouton dynamics have been performed in mature mice using axonal swellings as an indication of presynaptic terminals. However, during postnatal development, most synapses are not initially formed on dendritic spines, and axonal swellings are not prominent at the earliest stages of presynaptic development. Here, we have used cranial window implants and two-photon microscopy to perform time lapse imaging of synapse formation and elimination by identified mouse neocortical neurons in vivo. To examine synapse development from the earliest stages, presynaptic terminals were visualized by imaging fluorescently-tagged synaptophysin. To compare synapse formation and elimination throughout postnatal development, we imaged the same neurons during the period of intense synapse formation prior to the critical period, throughout the critical period, and after the end of the critical period.

Disclosures: T. Evans: None. **L. Bury:** None. **S.L. Sabo:** None.

Nanosymposium

576. Synapse Function in Development and Disease

Location: 147A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 576.03

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

Support: NIH R01DA024681

NIH R01MH101382

NIH P01NS048120

The Simons Foundation GC221194

Title: Distinct lineage-dependent structural and functional organization of the hippocampus

Authors: ***H. XU**[1], Z. HAN[2], P. GAO[1,3], S. HE[1], Z. LI[1], W. SHI[1,3], O. KODISH[1], W. SHAO[1,4], K. BROWN[1,3], K. HUANG[5], S.-H. SHI[1,3];
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Abstract: The hippocampus, as part of the cerebral cortex, is essential for memory formation and spatial navigation. Although it has been extensively studied, especially as a model system for neurophysiology, the cellular processes involved in constructing and organizing the hippocampus remain unclear. Here, we show that clonally related excitatory neurons in the developing hippocampus are progressively organized into discrete horizontal, but not vertical, clusters in the stratum pyramidale, as revealed by both cell type-specific retroviral labeling and mosaic analysis with double markers (MADM). Moreover, distinct from those in the neocortex, sister excitatory neurons in Cornu Ammonis 1 (CA1) region of the hippocampus rarely develop electrical or chemical synapses with each other. Instead, they preferentially receive common synaptic input from nearby fast-spiking (FS), but not non-FS, interneurons and exhibit synchronous synaptic activity. These results suggest that shared inhibitory input may specify horizontally clustered sister excitatory neurons as functional units in the hippocampus.

Disclosures: **H. Xu:** None. **S. Shi:** None. **P. Gao:** None. **S. He:** None. **Z. Li:** None. **W. Shi:** None. **O. Kodish:** None. **W. Shao:** None. **K. Brown:** None. **Z. Han:** None. **K. Huang:** None.

Nanosymposium

576. Synapse Function in Development and Disease

Location: 147A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 576.04

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

Support: Simons Foundation Autism Research Initiative Grant 274578

Title: The splicing factor Rbfox1 mediates the maturation and synaptic integration of cortical interneurons

Authors: *X. JAGLIN[1], B. WAMSLEY[1], H. XU[1], G. SALDI[2], B. RUDY[1], G. FISHELL[1]; [1]NYU Neurosci. Inst., New York Univ. Sch. of Med., New York, NY; [2]NYU Ctr. for Genomics and Systems Biol., NYUAD, Abu Dhabi, United Arab Emirates

Abstract: Cortical GABAergic interneurons provide inhibition that is crucial for the computational power of the cerebral cortex. This class of inhibitory neurons exhibit a striking diversity of morphological, electrophysiological and connectivity features that together define various interneuron subtypes. How this diversity is accomplished and how various interneuron types further integrate into the cortex during development is not fully understood. We are interested in understanding the mechanisms underlying the establishment of specific interneuron afferent and efferent connectivity. Developmentally regulated genetic programs are known to regulate interneuron fate and maturation, although, they cannot account for the whole array of interneuron types and connectivity patterns. Interestingly, recent work has shed light on the general requirement for neuronal activity in cortical interneuron migration, differentiation and possibly integration into circuits. However, the molecular connection between activity, gene expression regulation and interneuron integration into cortical networks remains to be elucidated. Activity-dependent alternative splicing (AS) of transmembrane proteins or ion channels has recently emerged as a potent post-transcriptional contributor to the dynamic regulation of cell-cell recognition and synaptic plasticity. Using RNA sequencing, we observed that similar functionally related genes are dynamically spliced in Parvalbumin (PV)-expressing basket and Somatostatin (SST)-expressing Martinotti cells during cortical network assembly. Therefore, we propose that AS may tailor the transcriptome to promote the integration of specific interneuron subtypes into developing cortical circuits. We recently identified the RNA binding protein, Fox-1 homolog (Rbfox1) to be developmentally enriched in the precursors that give rise to PV- and SST-expressing interneurons. Rbfox1 is an activity-regulated splicing factor that has been shown to modulate the alternative splicing of an array of ion channels, neurotransmitter receptors and transmembrane proteins, affecting neuronal excitability and potentially synaptogenesis. We are investigating whether Rbfox1 regulates the establishment of specific synaptic connectivity between cortical interneurons and excitatory neurons as well as the sub-cellular localization and stoichiometry of synapses.

Disclosures: X. Jaglin: None. **B. Wamsley:** None. **H. Xu:** None. **G. Saldi:** None. **B. Rudy:** None. **G. Fishell:** None.

Nanosymposium

576. Synapse Function in Development and Disease

Location: 147A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 576.05

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

Support: R01 MH067842

Title: The function of MET signaling in developing synaptic organization

Authors: *Z. XIE[1], K. EAGLESON[1,2], P. LEVITT[1,3];
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Abstract: The MET receptor tyrosine kinase, which is associated with autism spectrum disorders, influences cortical circuit function. The MET is expressed at high levels transiently during neocortical synaptogenesis, but the underlying mechanisms through which receptor dysfunction impacts cortical circuits are currently unknown. To address this knowledge gap, we performed experiments on the known MET/β-catenin protein interaction, focusing here on a putative role in presynaptic development. Co-immunoprecipitations, using either β-catenin or MET antibodies, validated the presence of the MET/β-catenin complex in neocortical synaptosomes at the peak of synaptogenesis (postnatal day 14). Two proteins that interact with β-catenin, N-cadherin and α-catenin, were not associated with MET, forming instead a separate complex with β-catenin. Following stimulation with hepatocyte growth factor (HGF), the ligand that activates the MET receptor, β-catenin is phosphorylated at tyrosine142 and dissociates from the MET/β-catenin complex. Simultaneously, there is an increase in the phosphorylated β-catenin/N-Cadherin complex and recruitment of synapsin I to interact with activated MET. Functional studies of cortical neurons in vitro show that MET activation promotes accumulation of synaptic vesicles that align with postsynaptic markers PDS-95 and Basoon. These data suggest a novel model whereby MET activation participates in the alignment of pre-postsynaptic elements that is necessary for normal maturation of functional synapses.

Disclosures: Z. Xie: None. **K. Eagleson:** None. **P. Levitt:** None.

Nanosymposium

576. Synapse Function in Development and Disease

Location: 147A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 576.06

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

Support: NIH 2 R56 NS063228-04

NIH R01 NS085993-01

Title: Torsin mediates primary envelopment of large ribonucleoprotein granules at the nuclear envelope for the egress of synaptically localized transcripts

Authors: *V. JOKHI[1], J. ASHLEY[2], J. NUNNARI[2], A. NOMA[3], N. ITO[4], N. WAKABAYASHI-ITO[4], M. MOORE[3], V. BUDNIK[2];
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Abstract: Recent studies have uncovered a novel, nuclear pore complex (NPC)- independent pathway of ribonucleoprotein (RNP) exit from the nucleus, within the context of synapse development. A central dogma in cell biology, established over half a century ago, is that all nucleo-cytoplasmic traffic occurs via NPCs. However, while studying a Wnt signaling pathway (Frizzled Nuclear Import (FNI) pathway), which is essential for synapse development in Drosophila, we discovered a novel mechanism by which large RNPs (mega-RNPs) exit the nucleus (Speese et al (2012) Cell 149:832). In this mechanism, megaRNPs bud through nuclear envelope (NE) membranes. Notably, this process is akin to the nuclear egress of herpes-type viruses (HSV), but was thought unique to these viruses. We now have compelling evidence that NE budding is an endogenous cellular process, which is likely hijacked by HSV. NE budding entails primary envelopment of viral capsids or megaRNPs by the inner nuclear membrane (INM), the scission of this envelope from the INM to create a membrane bound particle within the perinuclear space, which subsequently fuses with the outer nuclear membrane (ONM) to allow nuclear escape of the enclosed particle (or granule). However, the molecular machinery required to remodel the nuclear envelope during this process, was unknown. We have identified Torsin, a AAA-ATPase that in humans is linked to dystonia, as a major mediator of primary megaRNP envelopment during NE budding. In torsin mutants, megaRNPs accumulate within the perinuclear space and fail to reach synaptic sites, preventing normal synaptic protein synthesis, and thus proper synaptic bouton development. These studies begin to establish the cellular machinery underlying the exit of megaRNPs via nuclear envelope budding, offer an explanation to the “nuclear blebbing” phenotype found in dystonia models and provide a novel link between Torsin and synaptic phenotypes observed in dystonia.

Disclosures: V. Jokhi: None. **J. Ashley:** None. **J. Nunnari:** None. **A. Noma:** None. **N. Ito:** None. **N. Wakabayashi-Ito:** None. **M. Moore:** None. **V. Budnik:** None.

Nanosymposium

576. Synapse Function in Development and Disease

Location: 147A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 576.07

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

Support: NIH Javitz R37

Title: Regulation of Climbing fiber-purkinje cell synaptic pruning in a mouse model of SCA1

Authors: *E. A. LEATHLEY[1], M. INGRAM[1], H. Y. ZOGHBI[2], H. T. ORR[1];
[1]Univ. of Minnesota, Minneapolis, MN; [2]Baylor Col. of Med., Houston, TX

Abstract: Synaptic pruning is crucial for the development of functional neuronal circuits. The cerebellar Climbing Fiber (CF)-Purkinje cell (PC) synapse provides a simplified developmental system in which to study the synaptic pruning process. At birth each PC soma is innervated by multiple CFs. During the first 2-3 postnatal weeks in the rodent the supernumerary CFs are pruned away with a single remaining CF that then translocates up the PC dendritic tree to innervate a single PC. Developmental deficits in cerebellar CF-PC synapse pruning are found in mouse models of cerebellar dysfunction. We previously observed abnormal CF-PC synapse formation in an animal model of SCA1: ATXN1[30Q]S776D/+ transgenic mice (D30). These abnormal CF-PC synapses consist of perisomatic CF synaptic puncta that are indicative of a possible pruning deficit during postnatal cerebellar development. The mechanism by which this occurs is unknown, and the outcome of this developmental abnormality is largely unexplored. Pathogenic ATXN1 protein leads to the misregulation of multiple genes. Recent data have revealed that the D30 mice have significantly upregulated cholecystokinin (CCK) mRNA compared to WT animals during the critical period for CF synaptic pruning; a phenomenon that has been observed in other cerebellar mutant mice. Loss of CCK function in D30 mice decreases the number of abnormal CF-PC synapses in adult D30 animals, indicating that a CCK-mediated pathway is responsible for the alteration in CF-PC pruning. In situ hybridization studies revealed that CCK is likely upregulated in PCs of the cerebellum to elicit this effect. Our ongoing work aims at elucidating the pathway(s) altered by CCK to further mechanisms that regulate synaptic formation and pruning during postnatal development in the mouse.

Disclosures: E.A. Leathley: None. **M. Ingram:** None. **H.T. Orr:** None. **H.Y. Zoghbi:** None.

Nanosymposium

576. Synapse Function in Development and Disease

Location: 147A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 576.08

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

Support: NIMH Grant 1RO1MH097949-01

Autism Speaks #7359

Title: Using exogenous trafficking motifs to elucidate the mechanism by which autism associated PTEN point mutations result in loss of function

Authors: *C. J. FRICANO, T. DESPENZA, Jr., M. LI, M. R. WILLIAMS, B. W. LUIKART; Neurobio. and Physiol., Geisel Sch. of Med. At Dartmouth Col., Lebanon, NH

Abstract: Many growth factors exert their action through receptor tyrosine kinase receptors and activation of the PI3K, MAPK, and PLCγ pathways. Phosphatase and tensin homolog (PTEN), a known lipid phosphatase, acts as a negative regulator of this signaling by catalyzing the reverse reaction of PI3K via dephosphorylation of PIP3 to PIP2. This negatively regulates downstream AKT signaling resulting in decreased cellular growth and proliferation. PTEN knockdown or knockout in neurons results in a rapid onset of hypertrophy, as well as increases in pAKT, pMTOR, and pS6 signaling. PTEN is mutated in a subset of children with macrocephaly and autism; however, the mechanism by which these point mutations might alter PTEN function is unknown. To study this, we used viral-mediated molecular reconstitution of PTEN point mutations (H93R, F241S, D252G, W274L, and D326N) that have been identified in autism patients. We performed lentiviral knockout of endogenous PTEN with a virus that expresses Cre-recombinase into PTEN flx/flx mice, and simultaneously expressed full length and mutated PTEN protein. We have demonstrated that substitution of wild-type GFP-PTEN can rescue the neuronal hypertrophy observed with PTEN knockout. However,expression of these point mutations in vivo is not able to rescue this hypertrophy or increase in pS6, suggesting that they are loss of function mutations. In order to determine the mechanism by which these point mutations are loss of function, we have examined and manipulated the subcellular localization of these point mutations. Since wild-type PTEN is located to the cytoplasm, membrane, and nucleus, we sought to determine which of these locations is important for rescuing neuronal hypertrophy. D252G and F241S demonstrate a lack of nuclear localization, therefore, we have added SV-40 nuclear localization sequences to these mutations to determine if expression of these mutant forms of PTEN in the nucleus rescues their loss of function. We also cloned pleckstrin homology domains to the N-terminus of these mutations to target them to the plasma membrane. Finally, we demonstrated that lack of protein stability may contribute to the loss of

function of these ASD-associated point mutations. Thus, we conclude that H93R, F241S, D252G, W274L, and D326N confer PTEN loss of function via differing mechanisms regarding their subcellular localization and stability.

Disclosures: C.J. Fricano: None. **T. DeSpenza:** None. **M. Li:** None. **M.R. Williams:** None. **B.W. Luikart:** None.

Nanosymposium

576. Synapse Function in Development and Disease

Location: 147A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 576.09

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

Support: NIH Grant R01 MH097949

Autism Speaks: Pilot Grant #7359

Title: Hyperactivity of developing neurons lacking the autism-associated gene Pten results from increased excitatory synaptic drive

Authors: *M. R. WILLIAMS[1], J. LEE[2], G. B. RUSSO[2], D. R. RACINE[2], A. T. GULLEDGE[3], B. W. LUIKART[3];
[1]Physiol. & Neurobio., Dartmouth Med. Sch., Lebanon, NH; [2]Dartmouth Col., Hanover, NH; [3]Physiol. and Neurobio., Geisel Sch. of Med. at Dartmouth Col., Lebanon, NH

Abstract: The dual specificity lipid and protein phosphatase, Pten, is a negative regulator of the PI3K/mTOR pathway mutated in a subset of patients having ASD and macrocephaly. Pten depletion is associated with aberrant neuronal excitability, but the etiology is unclear. We designed retroviruses encoding a fluorescent reporter only (Control), or encoding a distinct fluorescent protein and Cre recombinase via a T2A motif (Pten KO). By co-injecting these particles to the dentate gyrus of neonatal, Pten-floxed mice we selectively infected newborn granule neurons, which then differed only in their Pten status. At increasing days post-injection, we generated hippocampal slices and analyzed how Pten KO altered developmental changes in of excitability. Neuronal hypertrophy occurred within 7 days; by 12.5 days, the mTOR- and activity- indicator, phosphorylated ribosomal S6, was elevated; by 16.5 days, levels of c-FOS were also up. By electrophysiology, we detected an increase in capacitance and a decrease in input resistance, but this resulted in developing Pten KO neurons requiring more current

injection to fire. By patch-clamp recordings, or by employing a novel retrovirus expressing GCaMP6s to image ensembles of cells, we found that Pten KO resulted in neurons firing at lower levels of perforant path stimulation. We discovered that Pten KO neurons developed a specific increase in the amplitude of evoked excitatory currents. To determine whether this was due to an increased number of synapses or was due to an increase in the amplitude of individual synapses, we isolated quantal-like evoked currents using strontium. We found a modest increase in the amplitude of individual events but a more robust change in the number of quanta per evoked current. This suggested Pten KO neurons develop more excitatory synapses. Using live 2-photon imaging we found that early in development, Pten KO neurons had more filopodial dendritic protrusions (potential precursors to mature spines), while later in development, Pten KO neurons had more mushroom spines (thought to be functional excitatory synapses). This proposed a morphological basis for increased sensitivity to excitatory input: a higher spine density. However, we also found Pten KO increased the size of the dendritic arbor. Mathematical modeling indicated that, collectively, the increase in: dendritic arborization, mushroom spine density, and quantal-like amplitude, completely accounted for elevated excitatory synaptic input in Pten KO neurons. Thus we conclude that, during development, isolated Pten knockout neurons dendritically recruit more excitatory synaptic inputs, resulting in a net increased activity in vivo.

Disclosures: **M.R. Williams:** None. **J. Lee:** None. **G.B. Russo:** None. **D.R. Racine:** None. **A.T. Gulledge:** None. **B.W. Luikart:** None.

Nanosymposium

577. Physiology of Glia-Neuronal Interactions

Location: 206

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 577.01

Topic: B.11. Glial Mechanisms

Support: McGill Faculty of Medicine Internal Studentship

Archimedes/Estonian Ministery of Education PhD scholarship

CIHR

Title: The dynamic extension of radial glial filopodia in response to neuronal activity contributes to synaptic maturation in the developing Xenopus retinotectal system

Authors: *M. SILD[1], M. VAN HORN[2], D. JIA[2], E. S. RUTHAZER[3];
[1]Psychiatry, [2]Neurosci., McGill Univ., Montreal, QC, Canada; [3]Neurosci., Montreal Neurolog. Institute, McGill Univ., Montreal, QC, Canada

Abstract: Radial glia are mainly known for their role as migratory scaffolds for neuroblasts. However, recent reports about radial glia-like cells like Bergmann glia and Muller glia have revealed the importance of such cells in synapse formation and maintenance. It is not clear how classic radial glia participate in circuit formation. Using two-photon microscopy, we have observed that radial glial filopodia in the living Xenopus laevis tadpole brain are highly dynamic. Radial glial filopodia respond to blockade of neuronal N-methyl-D-aspartate (NMDA) receptor activation by reducing their motility rate. Here we demonstrate that glial cGMP-dependent protein kinase PKGI, a likely upstream regulator of Rho GTPases, mediates this neural activity-dependent change in glial motility. The motility of glial filopodia can also be suppressed by overexpressing constitutively active RhoA or dominant negative Rac. Expression of small GTPases in radial glia to inhibit their motility suppressed the normal maturation of synaptic contacts onto neighboring tectal neurons. In conclusion, the PKGI-dependent motility of radial glial filopodia may participate in the functional maturation of excitatory synapses in the developing retinotectal system.

Disclosures: **M. Sild:** None. **M. Van Horn:** None. **D. Jia:** None. **E.S. Ruthazer:** None.

Nanosymposium

577. Physiology of Glia-Neuronal Interactions

Location: 206

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 577.02

Topic: B.11. Glial Mechanisms

Support: NIH Grant EB00415

NIH Grant DK35124

NIH Grant EY13574

NIH Grant DK86125

NIH Grant DK72517

Guthy-Jackson Charitable Foundation

Title: Super-resolution optical imaging of aquaporin-4 arrays in astrocyte foot processes

Authors: *A. J. SMITH, A. S. VERKMAN;
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Abstract: The water channel aquaporin-4 (AQP4) forms supramolecular aggregates (orthogonal arrays) that polarize to astrocyte foot-processes adjacent to microvascular endothelia in the central nervous system. The small size and high density of AQP4 arrays at astrocyte end-feet precludes measurement of array size and interactions using conventional optical approaches. Here, we applied direct stochastic optical reconstruction microscopy (dSTORM) to image AQP4 arrays in 3-dimensions in antibody-stained paraffin sections of normal brain and spinal cord, and in brain tumor samples. Measurement of AQP4 aggregation state was validated by mathematical modeling and by imaging brain sections from AQP4 null mice virally transfected with M1-AQP4, an AQP4 isoform that does not form arrays, or with M23-AQP4, an AQP4 isoform that forms large arrays. Native AQP4 in mouse brain cortex was seen in arrays localized to astrocyte foot-processes adjacent to microcapillaries. Significant differences in the size and distribution of AQP4 arrays were found between: (i) end-feet and other regions of the plasma membrane of protoplasmic astrocytes in mouse cortex; (ii) distinct populations of astrocytes in mouse spinal cord and human brain cortex; and (iii) astrocytes and glioblastoma cells in human brain. Specific interactions between AQP4 arrays and the inwardly rectifying K+ channel (Kir4.1) were demonstrated in cultured astrocytes and brain slices using 2-color dSTORM. Our results establish point localization-based super-resolution optical microscopy to image AQP4 arrays and their interactions in astrocytes in tissue sections.

Disclosures: A.J. Smith: None. **A.S. Verkman:** None.

Nanosymposium

577. Physiology of Glia-Neuronal Interactions

Location: 206

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 577.03

Topic: B.11. Glial Mechanisms

Support: NIH Grant R01AG022508

NIH Grant R01EY021796

Title: *Drosophila* scotopic vision relies on a glutamate-gated chloride channel in glia

Authors: *P. GUO, Z. LUAN, C. KRAWIC, H.-S. LI;
Univ. Mass. Med., WORCESTER, MA

Abstract: A basic characteristic of scotopic vision in dim light conditions is the high sensitivity to light, which is believed to depend entirely on the density of visual pigments and the efficiency of signal transduction within rod photoreceptor cells. Using the Drosophila visual system as a model, here we demonstrate the importance of glial cells in the sensitivity of scotopic vision. In the fly eye, outer photoreceptors (R1-R6), which are equivalents to vertebrate rod cells, send axons to the lamina neuropil and form inhibitory synapses with the secondary sensory neurons large monopolar cells (LMCs). The neuronal components of lamina are separated into hundreds of cartridges by epithelial glial (EG) cells, which also extend thin layers of membrane into the central neuronal space of cartridge. A planar form of thin EG membrane, referred to as gnarl, interposes between glutamatergic amacrine cell (AC) and its postsynaptic partner T1 cell. We found that a glutamate-gated chloride channel GluCl is clustered in gnarl. When GluCl was downregulated specifically in glia through RNAi, the sensitivity of fly visual transmission in lamina was severely decreased. In vivo Calcium imaging revealed that the LMC response to light decrement was virtually abolished in the glial GluCl knockdown (KD) flies. In an optomotor behavioral assay, those KD flies failed to respond to moving visual cues under dim, but not bright, illumination. All visual defects of the KD flies were reproduced in a mutant of GluCl. We propose that glutamate released from interneurons such as AC may open GluCl channels on the surface of EG to supply Cl- ions for the inhibitory transmission between photoreceptor and LMC, which is essential for the fly vision in dim conditions. It is plausible that neurotransmitter-gated ion channels in perisynaptic glia also play a critical role in the facilitation of mammalian synaptic transmission.

Disclosures: **P. Guo:** None. **Z. Luan:** None. **C. Krawic:** None. **H. Li:** None.

Nanosymposium

577. Physiology of Glia-Neuronal Interactions

Location: 206

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 577.04

Topic: B.11. Glial Mechanisms

Support: Merit Review grant (NEUD-004-07F)

Title: Regulation of the neuroprotective transcription factor creb during glia-neuron interactions

Authors: *S. PUGAZHENTHI[1], L. QIN[3], R. BOUCHARD[2], T. CHONG[3];
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Abstract: Inflammatory mediators and reactive oxygen species released by activated microglia cause neuronal injuries in the brain. Injured neurons are known to send out distress signals to microglia to amplify and sustain chronic inflammation. But the molecular mechanism of these neuroglial communications is not clear. CREB, a nuclear transcription factor that plays a central role in neurotrophin signaling and cognition is downregulated during neuroinflammation. The main objective of this study was to determine if neuronal CREB regulation plays an active role in these neuroglial interactions. The following three coculture models consisting of human neuroprogenitor cell-derived neurons and microglia isolated from human fetal brain were employed: (i) Conditioned medium from Abeta-activated microglia was added to neurons to evaluate CREB function. (ii) Mixed cultures of neurons and microglia were exposed to Abeta oligomers. (iii) Neurons expressing different CREB constructs and microglia cultured on separate ACLAR membranes were placed side-by-side in the same dish to facilitate two-way communications. Conditioned medium from Abeta-treated microglia decreased the promoter activity of CREB-dependent BDNF by 45% in neurons. However, the same medium induced (140%) the promoter of CXCL10, a chemokine regulated by NF-kB and STAT-1 suggesting differential regulation of transcription factors. Western blotting showed parallel changes at the protein level. Neurotoxicity of Abeta oligomers increased in neuroglial mixed cultures as shown by caspase-3 assay. CREB-mediated expression of genes relevant to neuronal function and survival were determined following coculture of neurons and microglia on PEN membrane slides and laser capture microdissection of neurons. Decreases in the expression of CREB targets including BDNF, synapsin-1 were observed. Neurons expressing an active form of CREB were protected from Abeta-activated microglia as shown by immunostaining for the active form of caspase-3. We also made an interesting observation with the third coculture model when neurons and microglia cultured on separate ACLAR membranes were placed together in the same dish. The activation state of CREB modulated microglial activation. For example, when activated microglia were placed near neurons expressing wild type CREB, iNOS induction and Phox expression in the microglia decreased whereas the neurons expressing MCREB, a dominant negative CREB mutant, exacerbated microglial activation. Our findings suggest that neuronal CREB regulation plays an active role in the modulation of microglial behavior following coculture.

Disclosures: S. Pugazhenthi: None. **L. Qin:** None. **R. Bouchard:** None. **T. Chong:** None.

Nanosymposium

577. Physiology of Glia-Neuronal Interactions

Location: 206

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 577.05

Topic: B.11. Glial Mechanisms

Support: NIH Grant R01AG022508

NIH Grant R01EY021796

Title: *Drosophila* visual glia circulate extracellular K+ to facilitate inhibitory neuronal signaling

Authors: Z. LUAN, P. GUO, *H.-S. LI;
Neurobio., Univ. Mass. Med. Sch., WORCESTER, MA

Abstract: In the nervous system, electrical signals of neuron are carried by charged ions: Na^+ flux into neuronal dendrites depolarizes, and subsequent K^+ outflux through axonal membrane repolarizes the neuron. To maintain normal neuronal signaling, both K^+ and Na^+ need to be redistributed between neuronal environments for the restoration of ion gradients across the neuron membrane. A variety of *in vitro* studies have shown that glia such as astrocytes help to maintain low levels of extracellular K^+ concentration ($[K^+]_o$) through activities of Na^+/K^+-ATPase and inwardly rectifying K^+ channels (Kir). How glial cells transport K^+ *in vivo* and the impact of this glial function to neuronal signaling, however, are largely unknown. In this study, we use the *Drosophila* visual system as an *in vivo* model to study the importance of glial Kir channels to neuronal signaling. Fly photoreceptor neurons in the retina project axons to the first visual ganglion lamina, where they release histamine upon light stimulation to inhibit the secondary sensory neurons, i.e., large monopolar cells (LMCs). We found that a *Drosophila* Kir channel, Irk2, is expressed in surface and cortex glia that separate lamina from retina. When the Irk2 gene was deleted or specifically knocked down in surface and cortex glia, light stimulation evoked a larger $[K^+]_o$ increase with faster kinetics in both lamina and the second visual ganglion medulla. In contrast, the basal level of $[K^+]_o$ in the retina was significantly lower than wild type. Probably due to the excessive increase of $[K^+]_o$, laminar LMCs were excited instead of inhibited by light, as revealed by Ca^{2+} imaging in axon terminals of LMC. In electroretinogram (ERG) recordings, Irk2 deletion and knockdown flies showed abnormal, light-stimulated electric oscillation and an overshoot in the recovery phase of light response, suggesting signaling defects in additional visual neurons. Based on electron microscopy and temporally controlled Irk2 knockdown experiments, the abnormalities in neuronal signaling are not attributed to any developmental or morphological defect. Our data indicate that *Drosophila* visual glia circulates extracellular K^+ through Irk2 channels between lamina and retina, and that this function of glial Kir channel is essential for normal visual signaling. We propose that non-visual glial network in mammals also circulate K^+ between different brain regions to facilitate, at least inhibitory, neuronal signaling.

Disclosures: **Z. Luan:** None. **H. Li:** None. **P. guo:** None.

Nanosymposium

577. Physiology of Glia-Neuronal Interactions

Location: 206

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 577.06

Topic: B.11. Glial Mechanisms

Title: Intracellular trafficking, matrix association and function of VEGF in astroglial cells

Authors: K. EGERVARI[1], G. POTTER[1], P. SALMON[1], B. WEHRLE-HALLER[2], M. L. GUZMÁN-HERNÁNDEZ[3], T. BALLA[3], *J. Z. KISS[1];
[1]Dept Neurosci, Univ. Geneva Med. Sch., Geneva 4, Switzerland; [2]Dept. Cell Physiol. and Metabolism, Univ. Geneva Med. Sch., Geneva 4 1211, Switzerland; [3]NICHD, Natl. Inst. of Hlth., Bethesda, MD

Abstract: Vascular endothelial growth factor (VEGF), a well-known regulator of neurovascular remodeling following cerebral lesions, is a major factor by which reactive astrocytes exert widespread effects on their microenvironment. In the last decade much have been learnt about VEGF functions in endothelial cells, while other possible targets of the growth factor have also been proposed. However, little is known about the intracellular trafficking, mode of secretion and matrix interactions of VEGF in the “source” cells. Here, we generated a VEGF::GFP fusion protein to follow the distribution of VEGF165 during its trafficking in primary astrocytes and COS7 cells. We found that while it follows the endoplasmic reticulum-Golgi pathway, biologically active VEGF::GFP forms dimers and becomes glycosylated similarly to wild type. The secretion of VEGF::GFP occurs even at 19 °C and shows a Ca2+- and PKC-induced increase, suggesting that it follows the regulated secretory pathway during its post-Golgi trafficking. As we explored the expression of VEGF::GFP in polarized primary astrocytes in an in vitro wound healing assay, VEGF::GFP appeared to follow a vectorial distribution and dynamically accumulated on the extracellular surface behind the leading edge. Moreover, electron microscopic analysis revealed that extracellular VEGF::GFP remains associated with discrete areas of the cell membrane and is accumulated in caveolae as well as on shedding microvesicles. This particular localization overlaps with active focal/fibrillar adhesions (FB), where VEGF::GFP co-localizes with fibronectin. Extracellular matrix-bound VEGF::GFP is endocytosed by astrocytes at specialized cellular regions followed by rapid degradation. Finally, with fluorescence recovery after photobleaching (FRAP) experiments we show that integrin

turnover is decreased in FBs associated to cell-derived VEGF, raising the possibility of autocrine regulation of glial functions. Together, these findings not only have strong implications for understanding focal coordination of vascular remodeling by astroglia derived VEGF, but also shed light on a self-regulatory role of VEGF in reactive astrocytes.

Disclosures: **K. Egervari:** None. **G. Potter:** None. **P. Salmon:** None. **B. Wehrle-Haller:** None. **M.L. Guzmán-Hernández:** None. **T. Balla:** None. **J.Z. Kiss:** None.

Nanosymposium

577. Physiology of Glia-Neuronal Interactions

Location: 206

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 577.07

Topic: B.11. Glial Mechanisms

Support: ANR 12-BSV4-0013-01

Title: The role of connexin 30 in sleep homeostasis

Authors: ***X. LIU**[1], J.-M. PETIT[2], A.-C. BOULAY[1], M. COHEN-SALMON[1], P. MAGISTRETTI[2], C. GIAUME[1];
[1]Collège De France, Paris, France; [2]Brain Mind Institute, EPFL, Lausanne, Switzerland

Abstract: Astrocytes are important modulators of many brain functions. Recently, astrocytes have been shown to regulate sleep homeostasis through vesicular release of ATP which contributes to adenosine accumulation in the brain. Besides mechanisms at the cellular level, astrocytes might also act on a network level to influence sleep homeostasis: astrocytes form highly interconnected networks via gap junction channels, which are constituted by connexins (Cxs), mainly Cx30 and Cx43. We observed that mRNA expression of Cx30 but not Cx43 is enhanced after 6-hour sleep deprivation in both the cortex and the hippocampus of mice. Also, gap junctional communication among astrocytes is increased by sleep deprivation in wild type (WT) mice but in Cx30 knockout (Cx30-KO) mice. Thus we investigated the potential role of Cx30 in sleep homeostatic regulation. We measured the sleep-wake behavior of the mice and found that compared to WT mice: 1) spontaneous locomotor activity is decreased in Cx30-KO mice by 27% during the dark and by 38% during the light period; 2) the number of stimulations (by adding new nesting material or new objects to the cage) needed to maintain Cx30-KO mice awake during 6-hour gentle sleep deprivation is increased by 49%; 3) slow wave sleep duration is increased during 6-hour instrumental sleep deprivation by 50% in Cx30-KO mice monitored

by electroencephalography and electromyography recordings (preliminary data). To probe the possible mechanisms underlying the sleep phenotypes of the Cx30-KO, the mRNA levels of genes related to brain energy metabolism and neurotransmitter and neuromodulator systems involved in sleep-wake regulation were measured by qPCR in different brain structures. We found in multiple brain structures significant decreases in mRNA levels of the following genes: the neuronal monocarboxylate transporter (MCT2) by 8 to 12%, the lactate dehydrogenase A by 14 to 20%, the glycogen phosphorylase by 9 to16% and the protein targeting to glycogen by 13 to 21%. Furthermore, the orexin receptor 2 is downregulated in the hypothalamus by 24%. These results suggest that Cx30 plays an important role in wakefulness during periods of high sleep pressure, possibly by supporting the high metabolic demand and the proper function of orexinergic transmission.

Disclosures: X. Liu: None. **J. Petit:** None. **A. Boulay:** None. **M. Cohen-Salmon:** None. **P. Magistretti:** None. **C. Giaume:** None.

Nanosymposium

577. Physiology of Glia-Neuronal Interactions

Location: 206

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 577.08

Topic: B.11. Glial Mechanisms

Support: NSF IGERT CMMB 0965918

NSF Grant IOS-1354913

Title: Heterogeneity of astrocyte morphology and function in hippocampal dentate gyrus

Authors: *G. NASERI KOUZEHGARANI[1], M. U. GILLETTE[2];
[1]Neurosci. Program, [2]Dept. of Cell and Developmental Biol., Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: Astrocytes play significant roles in regulation of neuronal activity with respect to neurogenesis, learning and memory, and synaptic plasticity. Previous studies have shown that there are two morphologically distinct populations of astrocytes in the CA1 and CA3 layers of the rat hippocampus with mutually exclusive functional properties. One group exclusively expresses glutamate transporters and cells are coupled through gap junctions. The other group only expresses AMPA-type glutamate receptors and lacks gap-junction coupling. However, little

is known about differences in astrocytic structural and functional dynamics in the hippocampal dentate gyrus. Here we show that two subpopulations of astrocytes with distinct morphological and physiological properties reside in the granular layer of the dentate gyrus. Using whole-cell patch clamp recording and dye-filling techniques, we found that injection of a single astrocyte with biocytin leads to visualization of astrocyte networks consisting of 20-50 cells that are linked through gap junctions. These coupled cells show a linear response of voltage in response to current injection. Conversely, upon injection of the dye into the other group of astrocytes only the single patched cell was labeled, indicating it lacks gap junctions. Additionally, these cells exhibit non-linear voltage changes to current injection, specifically an inward rectifying profile. Furthermore, the size of the soma in the gap junction-coupled astrocytes is approximately twice as large as the non-coupled cells. Our results demonstrate that the two subtypes of astrocytes within the hippocampal granular layer of the dentate gyrus are comprised of distinct structural and physiological populations with different functional dynamics through expression of either glutamate transporters or receptors. These two populations of astrocytes could have different functions in regulating synaptic plasticity and neurogenesis with potential links to learning and memory.

Disclosures: G. Naseri Kouzehgarani: None. **M.U. Gillette:** None.

Nanosymposium

577. Physiology of Glia-Neuronal Interactions

Location: 206

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 577.09

Topic: B.11. Glial Mechanisms

Support: Lowy Medical Research Institute

National Health and Medical Research Council Australia, APP1028393

National Health and Medical Research Council Australia, APP1050373

Ophthalmic Research Institute of Australia

Title: Changes in retinal metabolomics after selective Müller cell ablation

Authors: *W. SHEN[1], J. DU[2], S. LEE[1], S. H. CHUNG[1], L. ZHU[1], C. D. RAE[3], J. B. HURLEY[2], M. C. GILLIES[1];
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Abstract: Background and Aim: Glucose metabolism plays a critical role in retinal health and disease. Müller cells are one of the major sites of glucose metabolism in the retina where they provide critical metabolites such as lactate to fuel retinal neurons. The aim of this study was to analyze changes in retinal metabolomics in a novel transgenic model in which selective Müller cell ablation leads to photoreceptor degeneration, vascular leak and intraretinal neovascularization. **Methods:** Transgenic mice carrying a portion of the regulatory region of the retinaldehyde binding protein 1 (Rlbp1) gene and the Cre-Estrogen Receptor (ER) construct were crossed with Rosa-DTA176 mice for selective Müller cell ablation after tamoxifen (TMX)-induced Cre recombination. Mice carrying the DTA176 gene but not the Rlbp1-CreER construct were treated with TMX as controls. Retinas were collected 5 weeks after Müller cell ablation to profile changes in the retinal metabolomics. We also studied changes in U-^{13}C-glucose derived metabolic intermediates using retinas collected 45 minutes after intraperitoneal injection of U-^{13}C-glucose (500mg/kg BW). **Results:** A total of 411 metabolic mediators were detected in the mouse retina, including 335 compounds of known identity and 76 of unknown identity. Müller cell ablation induced significant reduction of lactate and glycerate, suggesting a disruption in the glycolysis pathway. We also found changes in lipid metabolism and sphingolipids, reflecting disruption in mitochondrial metabolism and tissue membrane structure. Other metabolic changes included decreased levels of branched chain amino acids, indicating tissue remodeling, and a disruption in protein synthesis. We also found reduced metabolites in nucleic acid catabolism and polyamines. Analysis of U-^{13}C-glucose derived metabolites after Müller cell ablation showed changes that were consistent with the findings revealed by metabolic profiling. Müller cell ablation markedly reduced the levels of labeled glycolytic intermediates including dihydroxyacetone phosphate, glyceraldehyde 3-phosphate, 3-phosphoglyceric acid, phosphoenopyruvate, pyruvate and lactate, TCA cycle intermediates including citrate, alpha-ketoglutarate, succinate, fumarate and malate, and amino acids including alanine, glutamate, glutamine, GABA and aspartate. **Conclusion:** We have identified changes in the metabolic profile caused by induced Müller cell disruption that may contribute to photoreceptor degeneration and tissue remodelling in retinal disease.

Disclosures: W. Shen: None. **J. Du:** None. **S. Lee:** None. **S.H. Chung:** None. **L. Zhu:** None. **C.D. Rae:** None. **J.B. Hurley:** None. **M.C. Gillies:** None.

Nanosymposium

577. Physiology of Glia-Neuronal Interactions

Location: 206

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 577.10

Topic: B.11. Glial Mechanisms

Support: Foundation for Science and Technology (FCT, Portugal)

Calouste Gulbenkian Foundation

Howard Hughes Medical Institute

Title: Long-term memory is defective in mice with impaired astrocytic calcium signaling

Authors: *A. PINTO-DUARTE[1], A. J. ROBERTS[2], K. OUYANG[3], J. CHEN[4], T. J. SEJNOWSKI[1];
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Abstract: The structural disposition of astrocytes around synapses places them at the core of information transmission. These glial cells communicate through type 2 IP3 receptor (IP3R2)-dependent calcium oscillations, which are thought to drive the release of neuroactive substances, such as glutamate, ATP or D-serine. When compared to wild type mice, IP3R2 KO mice exhibit impaired hippocampal LTP following theta-burst stimulation of the alveus, presumptively due to a reduction in astrocyte-derived glutamate levels (Navarrette et al., 2012). Our present goal was to investigate if the absence of IP3R2 in vivo led to alterations upon cognitive behaviors, particularly those requiring activation of the hippocampal formation. We found that IP3R2 KO mice presented significant deficiencies in several memory domains as compared to their wild type littermates, including abnormal spatial memory and impaired capacity to recognize novelty. We further found evidence that said deficiencies were specific to behavioral tasks that depended upon learning and memory employment. These data are in support of an important role of IP3R2-mediated astrocyte calcium signaling for information storage and its manipulation in the brain.

Disclosures: **A. Pinto-Duarte:** None. **A.J. Roberts:** None. **K. Ouyang:** None. **J. Chen:** None. **T.J. Sejnowski:** None.

Nanosymposium

577. Physiology of Glia-Neuronal Interactions

Location: 206

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 577.11

Topic: B.11. Glial Mechanisms

Support: NIA R01AG037599

VA Merit Review

Title: Real time changes of extracellular lactate levels during synaptic train stimulation in rat hippocampal slices

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Abstract: Synaptic stimulation induces an increase in oxygen utilization, NADH oxidation, and glucose utilization by neurons and astrocytes. In vivo data suggest that during activation, increased lactate release, likely from astrocytes, may be coupled with lactate utilization by neurons. However, limited data are available regarding the kinetics of lactate release in direct relationship to oxygen utilization during neuronal activation in brain tissue. Therefore, we developed an enzyme-based microelectrode to measure extracellular lactate in the tissue with high temporal resolution, to analyze extracellular lactate levels in rat hippocampal slices. We measured either changes in lactate levels or tissue Po2 (nadir) and fEPSP simultaneously in area CA1 in interface hippocampal slices from 8 weeks old F344 rats (10 mM glucose, 400 μm, 36 °C, 95 % O2), during synaptic stimulation (10 Hz x 25-90 s) and under low glucose condition. During 25 s stimulation, lactate increased in the stratum radiatum and continued to rise for 12.5 ±1 s after the stimulation ended. The extracellular lactate transient was proportional to increasing stimulation intensity, ranging from 1.2 ± 0.3 mM to 3.04 ± 0.3 mM (n=4), with 50% and 90% of maximal stimulation intensity, respectively. Parallel to the lactate increase, tissue Po2 continued to drop until the end of the stimulation indicating persisting oxidative metabolism simultaneous with the lactate rise. In contrast, with prolonged stimulation (90 s) lactate transients peaked after ~ 53 s, demonstrating an average lactate increase of 2.7 ± 0.5 mM (at 70% of maximum intensity), but then declined to a lower plateau level that was maintained until the end of the stimulation. In both 25 s and 90 s trains, tissue lactate levels recovered to baseline at the end of the stimulation within ~ 80 s. Glucose deprivation (40 min) decreased fEPSP and lactate levels by 4 ± 1 mM. In addition, 0 glucose completely inhibited the stimulus-induced rise in lactate. These results confirm that during intense synaptic stimulation the rate of glycolysis (as indicated

by the excess lactate release into the extracellular space) transiently exceeds the rate of oxidative phosphorylation despite persistent oxygen utilization. In contrast, the maintained (but lower) lactate plateau during prolonged (90 s) trains suggests that over time cellular lactate uptake increases. Though, the absolute levels of lactate could not be calibrated with these electrodes, the dynamic increase with train stimulation and the significant decrease with zero glucose indicate that the extracellular lactate pool is a rapidly fluctuating reserve for interchange between astrocytes and neurons.

Disclosures: **F. Galeffi:** None. **M. Messerli:** None. **G. Katul:** None. **D.A. Turner:** None.

Nanosymposium

577. Physiology of Glia-Neuronal Interactions

Location: 206

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 577.12

Topic: B.11. Glial Mechanisms

Support: British Heart Foundation Grant FS/13/5/29927

Title: Purinergic signalling in the nucleus tractus solitarius controls hypoxic ventilatory responses in awake rats

Authors: ***P. S. HOSFORD**[1], M. LI[2], M. FIGUEIREDO[3], A. GOURINE[2], S. KASPAROV[3], N. MARINA[1];
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Abstract: Brainstem astroglial cells play a fundamental role in the control mechanisms of central respiratory chemosensitivity. In the ventral aspect of the medulla oblongata, astrocytes are functionally specialized to monitor physiological increases in $PCO_2/[H^+]$ (Gourine et al., 2010, Science 329:571) whilst in the dorsal aspect, astrocytes residing in the nucleus tractus solitarius (NTS) are activated in response to systemic hypoxia (Tadmouri et al, 2014, J. Neurosci. Res. 92:627). Here, we investigated whether NTS astrocytes are involved in the modulation of central respiratory activity as well as in the changes in respiratory activity induced by exposure to acute hypoxia. NTS astrocyte activation was monitored in anesthetized and artificially ventilated rats transduced to express a genetically encoded Ca^{2+} indicator (Case12) via an adenoviral vector with enhanced glial fibrillary acidic protein (GFAP) promoter. $[Ca^{2+}]_i$ elevations of 53±8% (based on 5 regions of interest) were evoked in response to systemic

hypoxia (10% O_2 inspired air for 2 min). To explore the role of purinergic gliotransmission in the control of breathing, a potent ectonucleotidase, transmembrane prostate acidic phosphatase (TMPAP) was expressed in the NTS for facilitated breakdown of extracellular ATP. Despite increased ventilation at rest in awake freely moving rats expressing TMPAP (82±9µl g^{-1} min^{-1}; n=8) c.f. GFP control animals (50±4 µl g^{-1} min^{-1}; n=6), there was no significant difference in the ventilatory response to hypoxia. Increased ventilation at rest in TMPAP expressing rats was reversed after systemic treatment with adenosine 1 receptor antagonist DPCPX (1mg kg^{-1}, i.p) and in these conditions, TMPAP expressing animals showed reduced ventilatory response to hypoxia of 8±12 µl g^{-1} min^{-1} (n=6) c.f control animals; 43±5 µl g^{-1} min^{-1} (n=5). This suggests that increased ventilation at rest was due to accumulation of adenosine following ATP breakdown and the absence of adenosine receptor activation unmasks the inability of the respiratory network to increase its activity due to the lack of extracellular ATP. The above data confirms that NTS astrocytes are activated by systemic hypoxia and further suggests that ATP gliotransmission contributes to the respiratory response to hypoxia.

Disclosures: P.S. Hosford: None. **M. Li:** None. **M. Figueiredo:** None. **A. Gourine:** None. **S. Kasparov:** None. **N. Marina:** None.

Nanosymposium

577. Physiology of Glia-Neuronal Interactions

Location: 206

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 577.13

Topic: B.11. Glial Mechanisms

Support: National Researc Foundation of Korea (NRF) grant 2011-0028319

Title: A novel type of neuronal death including ectopic mitochondrial calcification occurs during *in vitro* culture of rat hippocampal slices

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Abstract: Calcium precipitation is associated with impaired calcium homeostasis in several neuropathological disorders including brain ischemia, but the exact mechanisms of calcification need to be clarified. To investigate more detailed mechanisms causing ectopic calcification, we

used organotypic hippocampal slice culture (OHSC) because OHSC experiences trauma, cell death, and deafferentation from extrahippocampal regions during slice preparation and shares many of similarities with glial response seen in vivo condition with well-preserved three-dimensional configuration of the hippocampus. Temporal analysis was carried out, using Alizarin Red S staining for calcium precipitation and propodium iodide (PI) staining for evaluation of cell death, and immunohistochemistry for determination of cell type. Ultrastructural analysis of cell death and calcification was conducted using TEM and energy-dispersive X-ray spectroscopy (EDS). Despite the lack of PI staining, significant calcification was observed over the hippocampal pyramidal cell layer 6 days after preparation of OHSC. Ultrastructural analysis revealed that a number of dead neurons were randomly distributed among the normal pyramidal cells. These dead or dying neurons showed conserved conformations of nuclear and cytoplasmic membranes, but contained calcified granules, which had double-membrane structures and cisternae, suggesting mitochondrial origin. These neurons retained the compacted ultrastructure, even after majority of mitochondria being calcified. Despite the neuronal cell death, hardly any phagolysosome-containing microglial cells can be observed. Instead, the majority of dying cells were encircled by astrocytes, which contained profoundly dystrophic mitochondria and cytoplasmic vacuoles or inclusions of mixed electron density. In summary, our results demonstrate the following. First, a novel type of neuronal cell death, which is distinct from either necrosis or apoptosis, occurs in OHSC. Second, the dead neurons retained the compact ultrastructure, but underwent ectopic mitochondrial calcification. Third, these neurons were encircled and were eventually engulfed by astrocytes, but not by microglia. Thus, these data indicate that the special type of neuronal cell death occurs in an environment that is either apparently undamaged or excitotoxic, suggesting that ectopic mitochondrial calcification can be considered as a kind of mitochondrial adaptation to reduce free calcium ions in the neurons. This study was supported by the Mid-career Researcher Program through the National Research Foundation of Korea (NRF) grant funded by the MEST (2011-0028319).

Disclosures: **T. Riew:** None. **Y. Shin:** None. **J. Park:** None. **H. Kim:** None. **M. Lee:** None.

Nanosymposium

577. Physiology of Glia-Neuronal Interactions

Location: 206

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 577.14

Topic: B.11. Glial Mechanisms

Support: National Science Foundation Graduate Research Fellowship

ARCS Foundation Scholar Award

University of Arizona

Title: Physiology of *Drosophila* astrocyte-like glial cells: Intrinsic properties and response to channelrhodopsin-mediated neuronal activity

Authors: *S. E. MACNAMEE, L. P. TOLBERT, L. A. OLAND;
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Abstract: The *Drosophila* CNS contains a glial subtype that morphologically resembles vertebrate astrocytes and is associated with the synaptic neuropil. We have collected the first recordings from these astrocyte-like glial cells. They exhibit depolarization-activated currents and respond to optogenetically-mediated neuronal activity. The resting potential of larval ventral nerve cord astrocyte-like glia is -70mV, membrane resistance 50MΩ, membrane capacitance 140pF, and time constant 2.5ms (n=20 cells). An outward current activates at -40mV, peaks at 20ms, and then persists at half of peak amplitude for the duration of the voltage step. When returned to V_{rest}, the cells display inward tail currents, which peak at 10ms and are extinguished after 100ms. Pharmacological dissection suggests this voltage-gated current comprises potassium- and calcium-sensitive components. To examine neuron-glia interactions, channelrhodopsins (ChR2-H134R) were expressed in a subset of neurons and exposed to a 250ms optical stimulus. When clamped at -70mV, astrocyte-like glial cells always respond with a fast inward current that terminates at the offset of neuronal activity. In some cases, after termination of the optical stimulus, the glia display an outward current with slower kinetics. The fast inward current is abolished in the presence of 0 Ca^{++} and 1mM Cd^{++}, or in the presence of TTX, leading us to conclude that this current is linked to neurotransmitter release from the primary ChR2-expressing neuronal population. Unexpectedly, the 0 Ca^{++}/Cd^{++}-containing bath not only blocks the inward current but also induces a slow outward current, whether the outward current sometimes seen at the offset was, or was not, initially present. We varied the optical stimulus duration from 5ms to 5s, and found that the amplitude of the inward current grew with increasing stimulus duration up to 500ms, at which point neuronal firing rates display adaptation. When glial-cell holding potential was varied between -50mV and -100mV, the inward current amplitude increased with membrane hyperpolarization, arguing against its identity as an inward potassium current, and leaving open the possibility that this glial response is mediated by neurotransmitter receptors and/or transporters. The amplitude of the outward current at stimulus offset does not correlate with holding potential. These observations establish *Drosophila* as a model system for future exploration of the molecular/genetic underpinnings of intrinsic properties and neuron-to-astrocyte communication.

Disclosures: S.E. Macnamee: None. **L.P. Tolbert:** None. **L.A. Oland:** None.

Nanosymposium

578. Tauopathy: Molecular Pathogenesis and Experimental Therapy

Location: 152A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:00 PM

Presentation Number: 578.01

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

Support: DZNE

MPG

Tau Consortium

Title: The MAPT p.A152T mutation leads to excitotoxicity in a brain slice model of progressive supranuclear palsy (PSP)

Authors: *J. M. DECKER[1], L. KRÜGER[1], E. MANDELKOW[1,2,3], E.-M. MANDELKOW[1,2,3]; [1]DZNE, Bonn, Germany; [2]Caesar Res. Ctr., Bonn, Germany; [3]Max-Planck-Institute for Neurolog. Res., Hamburg Outstation, c/o DESY, Germany

Abstract: We investigated the pathophysiological consequences of human full-length Tau with the rare point mutation A152T (hTau40/A152T, related to PSP) in transgenic mice and cultured organotypic hippocampal slices. These mice show strong Tau pathology including hyperphosphorylation, aggregation, neuronal loss and behavior deficits (see accompanying abstract Sydow et al.). In slices from hTau40/A152T transgenic mice we detected early Tau phosphorylation at the KXGS motifs and at the PHF1 phospho-site. Tau was found in both presynaptic and somato-dendritic compartments including dendritic spines. A further anatomical observation was pronounced mossy fiber sprouting which is a key indicator for increased epileptiform activity. Indeed in such slices we detected pronounced epileptiform activity apparent in an enhanced burst and firing frequency. This seems to reflect the situation in PSP patients, where increased seizure susceptibility was reported (Nygaard et al., 1989, Neurology). To check for increased network excitation we next measured the glutamate content in the culture medium of hTau40/A152T slices. Glutamate started to rise early in culture followed by cytotoxicity. At the same time we detected elevated levels of intracellular calcium in CA3 neurons monitored with Fura-2 imaging. This increase of intracellular resting calcium was reflected in an enhanced activity dependent calcium influx after membrane depolarization both being sensitive to NR2B blockade. The glutamate increase was reduced to control levels by either the inhibition of neurotransmitter release or the blockade of voltage gated sodium

channels. In line with augmented extracellular glutamate and intracellular calcium concentrations, we observed enhanced basal synaptic transmission in the mossy fiber pathway of acute hippocampal slices from heterozygous 12-months old hTau40/A152T mice in the absence of changes in either short or long-term plasticity. Excitotoxic cell death in slices was ameliorated by long-term application of low-dose memantine and by administration of the ß-Lactam antibiotic ceftriaxone, which stimulates glutamate uptake via glial Excitatory Amino-Acid Transporter 2 (EAAT2). In summary, hTau40/A152T causes pronounced excitotoxicity mediated by the extrasynaptic NMDAR containing NR2B subunit due to an increase of extracellular glutamate, which is probably caused by enhanced presynaptic transmitter release.

Disclosures: J.M. Decker: None. **L. Krüger:** None. **E. Mandelkow:** None. **E. Mandelkow:** None.

Nanosymposium

578. Tauopathy: Molecular Pathogenesis and Experimental Therapy

Location: 152A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:00 PM

Presentation Number: 578.02

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

Support: Japan Society for the promotion of Science

the Tau consortium

Title: Analysis of *in vivo* turnover of tau in a mouse model of tauopathy

Authors: *K. YAMADA[1,2], T. K. PATEL[2], K. HOCHGRÄFE[3], T. E. MAHAN[2], H. JIANG[2], F. R. STEWART[2], E.-M. MANDELKOW[3,4,5], D. M. HOLTZMAN[2];
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Abstract: Aggregated forms of tau protein in structures such as neurofibrillary tangles are pathological hallmarks found in a set of neurodegenerative diseases called tauopathies. Impaired clearance of tau is hypothesized as one of the mechanisms, which results in age-dependent accumulation of misfolded tau and contributes to development of tau pathology. Nevertheless, the detailed characterization of *in vivo* turnover of specific tau species especially in the context of diseases has yet to be elucidated. In the present study, we examined *in vivo* turnover of tau in

a mouse model of tauopathy. We utilized mice over-expressing human tau with a ΔK280 mutation, one form of tau which leads to a familial form of frontotemporal dementia. Expression of ΔK280 tau in the mouse brain causes an age-dependent accumulation of pretangle tau (pro-aggregant mice). The expression of human tau in these mice can be specifically switched off by doxycycline. Here we report differences in the turnover of various forms of tau including intracellular soluble tau and insoluble tau, extracellular tau, and phosphorylated tau. Interestingly, the *in vivo* half-life of soluble tau turned out to be significantly longer than what was previously reported in most cell culture experiments. Biochemically soluble tau had a half-life of 9.9 days whereas insoluble tau had a much longer half-life. Remarkably, the half-life of extracellular tau in pro aggregant mice was 17.3 days, which is much longer than that seen in mice expressing a form of tau that does not aggregate (anti aggregant mice). In contrast to a previous assumption that phosphorylation may impair tau clearance, the half-life of certain phosphorylated forms of tau (pS202/pT205, pT231/pS235) was much faster (3-4 day) than total tau. In summary, our study reports differences in turnover rate of various tau species in a mouse model of tauopathy. The fact that certain tau species exhibit slower clearance may relate to the pathogenesis of tauopathy. In addition, the present in vivo study uncovers the novel interplay between aggregation and phosphorylation and clearance rate of intracellular as well as extracellular tau. *In vivo* turnover rate at the systemic levels also provides useful insights in regard to how to interpret therapeutic strategies aiming to lower tau levels from the perspectives of tau turnover. Acknowledgements: Japan Society for the promotion of Science (K.Y.) and the Tau consortium (D.M.H. and E.M.M.)

Disclosures: **K. Yamada:** None. **T.K. Patel:** None. **K. Hochgräfe:** None. **T.E. Mahan:** None. **H. Jiang:** None. **F.R. Stewart:** None. **E. Mandelkow:** None. **D.M. Holtzman:** None.

Nanosymposium

578. Tauopathy: Molecular Pathogenesis and Experimental Therapy

Location: 152A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:00 PM

Presentation Number: 578.03

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

Support: F30AG046088

P01AG017586

R01NS075487

T32GM008361

P30NS057098

Title: Targeting tau-mediated NMDA receptor hypofunction reverses deficits in a mouse model of frontotemporal dementia

Authors: *E. D. ROBERSON[1], B. A. WARMUS[2], D. R. SEKAR[2], E. MCCUTCHEN[2], G. D. SCHELLENBERG[3], L. L. MCMAHON[2];
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Abstract: Frontotemporal dementia (FTD) is rapidly progressive and lethal, with no disease-modifying treatments. It is known that tau mutations cause FTD, but the underlying neurobiology is undefined. We sought to identify how tau impairs neuronal network function in order to find potential treatment targets. Here, we address this question using a new mouse model expressing human tau with an FTD-associated mutation. We studied behavior, physiology, biochemistry, and neuropathology in several cohorts of mice at different ages. These mutant tau mice had abnormal repetitive behavior characteristic of FTD and synaptic deficits selectively in regions associated with FTD (ventral striatum and insula). There, mutant tau depleted PSD-95 and impaired synaptic localization of glutamate receptors, including NMDA receptors (NMDAR). Recordings from ventral striatum neurons revealed deficits in NMDAR-mediated synaptic transmission, resulting in impaired network activity. Pharmacologically targeting NMDAR hypofunction in vivo with cycloserine, an FDA-approved NMDAR co-agonist, reversed network impairments and repetitive behavior. These results indicate that mutant tau impairs NMDAR trafficking, causing NMDAR hypofunction in vulnerable brain regions, and that this process can be therapeutically targeted. These findings have important treatment implications, including the possibility of repurposing cycloserine for treating FTD.

Disclosures: E.D. Roberson: None. **B.A. Warmus:** None. **D.R. Sekar:** None. **E. McCutchen:** None. **G.D. Schellenberg:** None. **L.L. McMahon:** None.

Nanosymposium

578. Tauopathy: Molecular Pathogenesis and Experimental Therapy

Location: 152A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:00 PM

Presentation Number: 578.04

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

Title: Progressive tauopathy-dependent neurodegeneration in mice expressing an inducible tau transgene: Towards a new model of alzheimer's disease

Authors: *T. LI, J. ZHANG, A. LAU;
Pathology, The Johns Hopkins Univ., BALTIMORE, MD

Abstract: Effective therapy for Alzheimer's disease (AD), the most common form of dementia and a devastating illness fort he elderly, remains a great unmet need. For translational research and preclinical drug development, animal models that mimic the cardinal pathological features of AD will be critical. A major limitation of current mouse models is the lack of progressive AD-like neuropathology, especially the robust age-dependent neuronal loss in their brains. Here, we generated and characterized new mouse models that conditionally express the four repeat domain of human tau with the ΔK280 mutation (*Tau4R-ΔK280* mice). The *Tau4R-ΔK280* mice not only develop AD-like tau pathologies, but also recapitulate the age-dependent neuronal loss seen in AD. *Tau4R-ΔK280* mice exhibit age- and dosage- dependent hyperphosphorylated tau aggregation with ensuing deposition of tau tangles, neuronal loss and forebrain atrophy. Importantly, deficits in working memory in these mouse models occur during the early stages of development of tauopathy. As observed in cases of AD, reactive astrogliosis is associated with severe neuronal loss in *Tau4R-ΔK280* mice. Thus we have established a mouse model of tauopathy that mimic some salient features of AD. We anticipate that our *Tau4R-ΔK280* mice when crossbred to *APP* mice will create a mouse model of AD not only useful for studying disease mechanisms but amenable for pre-clinical drug screening and validation of novel therapies for AD.

Disclosures: **T. Li:** None. **J. Zhang:** None. **A. Lau:** None.

Nanosymposium

578. Tauopathy: Molecular Pathogenesis and Experimental Therapy

Location: 152A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:00 PM

Presentation Number: 578.05

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

Support: NIH Grant R01AG032279

NIH Grant U01AG046170

Alzheimer's Association Grant NIRG-10-173189

Title: A mechanism by which β-amyloid peptide and MARK/PAR-1 trigger abnormal metabolism and toxicity of microtubule-associated protein tau in a *Drosophila* model of Alzheimer's disease

Authors: *K. ANDO[1], Y. OHTAKE[1], A. MARUKO-OTAKE[1], M. SEKIYA[2], K. M. IIJIMA[2]; [1]Dept. of Neurosci., Thomas Jefferson Univ., Philadelphia, PA; [2]Dept. of Alzheimer's Dis. Research, Lab. of Genet. and Pathobiology, Natl. Ctr. for Geriatrics and Gerontology, Obu, Japan

Abstract: Tau is a microtubule-associated protein that localizes predominantly in the axons, where it regulates microtubule dynamics. Hyperphosphorylated tau is found in neurofibrillary tangles, the protein inclusions in the cytosol that are associated with a range of neurodegenerative diseases including Alzheimer's disease (AD). β-amyloid peptide (Aβ) is believed to play key roles in the pathogenesis of AD and lies upstream of the pathological changes in tau. Accumulation of abnormal tau has been suggested to cause neuron loss. However, the mechanism by which Aβ triggers tau abnormality is not fully understood. Elucidation of such mechanisms may reveal a strategy to block the cascades of pathological events leading to neuron loss in AD. By using a transgenic fly model of AD expressing human Aβ42 and tau in neurons, here we show that Aβ causes tau mislocation to the cytosol, which is stabilized by a tau kinase MARK/PAR-1. We found that Aβ42 expression caused a shift in the distribution of tau protein from the microtubule to the cytosol. In the presence of Aβ42, the levels of tau recovered in the microtubule fraction were reduced, and tau levels in the cytosol fraction were increased. In the normal neurons, RNAi-mediated knockdown of GSK3 increased tau binding to microtubules, indicating tau binding to microtubules is negatively regulated by tau phosphorylation by GSK3. We asked whether Aβ42 affects tau distribution via phosphorylation of tau at GSK3-target sites. Interestingly, knockdown of GSK3 did not block Aβ42-induced increase in tau levels in the cytosol. We found that Aβ42 disrupted microtubule integrity, which may underlie mislocation of tau species. These results suggest that Aβ42 creates a cellular environment in which tau species that are normally bound to microtubules mislocate to the cytosol. We previously reported that Aβ42 enhances tau toxicity, and tau phosphorylation at Ser262 is critical for this process. We found that Aβ42 increases the levels of tau phosphorylated at Ser262 via MARK/PAR-1. Interestingly, tau phosphorylation by MARK/PAR-1 increased tau levels. Blocking phosphorylation of tau at Ser262 via MARK/PAR-1 lowered the levels of tau in the cytosol fraction and reduced tau-induced neurodegeneration in the Aβ42 fly brain. These results suggest that tau species in the cytosol fraction may contribute to neurodegeneration in the presence of Aβ42. These results suggest that mislocation of tau to the cytosol and its stabilization by MARK/PAR-1 might be initial steps of abnormal metabolism of tau in the AD brain, and blocking these steps may be a possible strategy to prevent accumulation of patholollgical tau and neuron loss in AD.

Disclosures: **K. Ando:** None. **Y. Ohtake:** None. **A. Maruko-Otake:** None. **M. Sekiya:** None. **K.M. Iijima:** None.

Nanosymposium

578. Tauopathy: Molecular Pathogenesis and Experimental Therapy

Location: 152A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:00 PM

Presentation Number: 578.06

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

Support: Program for neurology research and discovery

A Alfred Taubman medical research institute

NIH DP3 DK094292

Title: Insulin resistance prevents AMPK-mediated tau dephosphorylation through increased AMPKSer485 phosphorylation

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Abstract: Metabolic syndrome (MetS) is a cluster of cardiovascular risk factors including obesity, diabetes and dyslipidemia. Insulin resistance (IR), a state of reduced responsiveness of target tissue(s) to normal circulating levels of insulin, is the central feature of MetS. It is now well established that MetS is a risk factor for Alzheimer's disease (AD). AMP-activated kinase (AMPK) is an evolutionarily conserved fuel-sensing enzyme and a key player in regulating energy metabolism. AMPK is activated during energy shortage (low ATP levels) and suppressed when energy is in surplus. This activation of AMPK is mediated by the phosphorylation of Thr172 by LKB1 or $CaMMK_{beta}$. Even though less studied, AMPK is also phosphorylated at Ser485 by Akt. $AMPK^{Ser485}$ phosphorylation is generally regarded to antagonize $AMPK^{Thr172}$ phosphorylation. Considering the important role of AMPK in energy metabolism, we contend that AMPK is the crucial link between IR and AD. In this report we examined the role of IR on the regulation of AMPK phosphorylation and AMPK-mediated tau phosphorylation. We found that $AMPK^{Ser485}$, but not $AMPK^{Thr172}$, phosphorylation is increased in the brains of db/db diabetic mice and high fat diet-fed obese mice, two mouse models of MetS. We and others demonstrated that tau phosphorylation is increased in these mouse models; therefore, we next examined the effect of AMPK activation on tau phosphorylation using a human cortical stem cell

line (HK-5320) and primary mouse embryonic cortical neurons. Treatment of cells with the AMPK activator, AICAR, induced AMPK phosphorylation at both Thr172 and Ser485 and also triggered tau dephosphorylation. When IR was mimicked *in vitro* by chronically treating the cells with insulin, AICAR specifically induced $AMPK^{Ser485}$, but not $AMPK^{Thr172}$, hyperphosphorylation whereas AICAR-induced tau dephosphorylation was inhibited. IR also resulted in the overactivation of Akt by AICAR treatment; however, preventing Akt overactivation during IR prevented $AMPK^{Ser485}$ hyperphosphorylation and restored AMPK-mediated tau dephosphorylation. Thus, our results demonstrate the following mechanism for the adverse effect of IR on AD pathology: IR chronic overactivation of Akt $AMPK^{Ser485}$ hyperphosphorylation inhibition of AMPK-mediated tau dephosphorylation. Together, these results show for the first time a contribution of IR-induced $AMPK^{Ser485}$ phosphorylation to the increased risk of AD in obesity and diabetes. This work is supported by the Program for Neurology Research & Discovery, A. Alfred Taubman Medical Research Institute and NIH DP3 DK094292

Disclosures: B. Kim: None. **P. Crystal:** None. **C. Backus:** None. **E.L. Feldman:** None.

Nanosymposium

578. Tauopathy: Molecular Pathogenesis and Experimental Therapy

Location: 152A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:00 PM

Presentation Number: 578.07

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

Support: NIH NS080881

CurePSP 468-08

Title: Zebrafish tauopathy models optimized for drug discovery and development

Authors: *E. A. BURTON[1], Q. BAI[2], Y. ZHOU[2], A. DUKES[2];
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Abstract: Progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) are common neurodegenerative diseases associated with prominent motor and cognitive abnormalities and a poor prognosis (median survival 6 - 8 years). Both diseases are characterized by neuronal loss and accumulation of abnormal aggregates of the 4-repeat isoform of the microtubule-associated protein Tau (4R-Tau) in neurons throughout the CNS. It is thought that

4R-Tau is central to the pathogenesis of these disorders, because mutations (e.g. P301L) in the MAPT gene encoding Tau can give rise to PSP and CBD phenocopies, and PSP and CBD are strongly associated with genetic variants at the MAPT locus. In order to address a critical barrier to progress in developing drugs to target Tau accumulation and its consequences in neurons in vivo, we have developed zebrafish Tauopathy models that are optimized for drug discovery, rapid comparative testing of putative therapeutic agents, and studies to identify novel therapeutic targets. We have made novel transgenic zebrafish that express human Tau conditionally at high levels in CNS neurons. These animals show: impaired survival; motor abnormalities demonstrable using automated video tracking in multiwell plates, with assay metrics suitable for drug discovery; oculomotor abnormalities typical of PSP; and histopathological changes representative of human disease, including somatodendritic accumulation of hyperphosphorylated Tau with immunoreactivity to a panel of antibodies used in the pathological diagnosis of human Tauopathies. Since these abnormalities occur rapidly over a short time course, these novel transgenic lines will be an invaluable tool for translational studies to develop new therapies for PSP and CBD, and potentially other Tauopathies such as Alzheimer's disease and chronic traumatic encephalopathy.

Disclosures: **E.A. Burton:** None. **Q. Bai:** None. **Y. Zhou:** None. **A. Dukes:** None.

Nanosymposium

578. Tauopathy: Molecular Pathogenesis and Experimental Therapy

Location: 152A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:00 PM

Presentation Number: 578.08

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

Support: Alzheimer's Art Quilt Initiative

BrightFocus Foundation

Coins for Alzheimer's Research Trust Fund

Title: Microglia and exosome-mediated spread of pathogenic tau in Alzheimer's disease

Authors: ***T. IKEZU**[1], H. ASAI[2], S. IKEZU[2], T. HAYDAR[3], B. WOLOZIN[1], S. KÜGLER[4]; [1]Pharmacol. and Neurol., [2]Pharmacol. and Exptl. Therapeut., [3]Anat. and Neurobio., Boston Univ. Sch. of Med., Boston, MA; [4]Ctr. for Nanoscale Microscopy and Physiol. of the Brain, Univ. Med. Göttingen, Göttingen, Germany

Abstract: The neurofibrillary tangle is a pathological hallmark of Alzheimer's disease (AD) and primarily consists of hyper-phosphorylated tau protein (pTau). pTau first appears in the entorhinal cortex in the presymptomatic stage, then gradually disseminates to the hippocampal region around the onset of clinical symptoms of AD. Halting this tau spread in the asympomatic stage is a promising therapeutic approach for AD. The exosome is a small vesicle of 50-100 nm in diameter, enriched in ceramide, and is suggested to contain neuropathogenic proteins, such as prion, α-synuclein, and recently tau proteins. A growing body of evidence suggests that microglia contribute to tauopathy-related pathogenesis in both human and animal models. We hypothesize that microglia transduce tau aggregates into nearby neuronal cells via exosomal secretion, and that inhibition of the exosome synthesis or secretory pathway reduces tau dissemination. We found that microglia efficiently phagocytose and secrete human tau aggregates in exosomes, which efficiently transduce tau aggregates in primary cultured mouse cortical neurons and induces accumulation of pTau. Moreover, we have created a novel mouse model exhibiting acute tau-spread by stereotaxic injection of adeno-associated virus expressing neuron-specific human mutant tau into the medial entorhinal cortex of mouse brain, which show spread of human tau to the granular cell layer of dentate gyrus at 28 days post injection. This tau spread was significantly suppressed by depletion of microglia or inhibition of neutral sphingomyelinase-2, which synthesizes ceramide and regulates exosome synthesis. These results demonstrate that microglia and exosomes play significant roles in spreading pathogenic tau in mouse brain. Our findings could lead to an entirely novel paradigm for delaying the progression of disease not only in AD but also other tauopathies such as FTD and chronic traumatic encephalopathy.

Disclosures: **T. Ikezu:** None. **H. Asai:** None. **S. Ikezu:** None. **T. Haydar:** None. **B. Wolozin:** None. **S. Kügler:** None.

Nanosymposium

579. Risk Factors for Neurodegenerative Diseases

Location: 150B

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 579.01

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

Support: NSFC Grant 30972461

NSFC Grant 81202291

Title: Prenatal marginal vitamin a deficiency facilitates alzheimer's disease pathogenesis

Authors: *J. ZENG[1,2], Q. CHEN[2], Z. FAN[2], H. JIANG[2], L. CHEN[2], J. CHEN[2], T. LI[2], W. SONG[1];
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Abstract: Deposition of amyloid β protein (Aβ) to form neuritic plaques in the brain is the pathological hallmark of Alzheimer's disease (AD). Mild or marginal vitamin A deficiency (MVAD) is a serious and widespread public health problem in pregnant women and children in developing countries. There has been increasing evidence for the involvement of vitamin A in AD pathogenesis. However, the role of vitamin A in the development of AD is not well defined. Our studies in an elderly population have shown that vitamin A deficiency (VAD) could enhance the risk to develop AD, and retinoic acid receptors (RARs) plays an important role in the amyloid β precursor protein (APP) metabolic pathway. To further examine vitamin A's effect on AD pathogenesis, we established a prenatal MVAD model in APP/PS1 double-transgenic AD model mice. Our study showed that MVAD significantly increases Aβ production by inhibiting ADAM10-mediated α-secretase cleavage and increasing β-secretase (BACE1) cleavage of APP. MVAD significantly increased neuritic plaque formation and aggravated spatial learning and memory deficits. Our findings provide a mechanistic explanation for the role of MVAD in AD pathogenesis and demonstrate the importance of retinoic acid signaling as a target for AD therapy.

Disclosures: J. Zeng: None. **W. Song:** None. **Q. Chen:** None. **Z. Fan:** None. **H. Jiang:** None. **L. Chen:** None. **J. Chen:** None. **T. Li:** None.

Nanosymposium

579. Risk Factors for Neurodegenerative Diseases

Location: 150B

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 579.02

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

Support: P01 AG030128-04

R01 AG035379-03

R03 AG039708-02

GUMC Grant Music for Mind

Title: Isoform and lipid dependent interaction of apolipoprotein E (apoE) with LRP1

Authors: *I. Y. TAMBOLI, G. REBECK;
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Abstract: The strongest genetic risk factor for late onset Alzheimer's disease (AD) is APOE. ApoE exists in humans as three major isoforms apoE2, apoE3 and apoE4. Being heterozygous or homozygous for the APOE-ε4 allele confers a 3 or 10 fold increase in AD risk, respectively. Binding of apoE to members of the low density lipoprotein receptor (LDLR) family and subsequent endocytosis are necessary for efficient uptake of lipoproteins by neurons, which supports neuronal maintenance, growth, and repair. Conditional forebrain knockout mice which do not express one of the LDLR family member LRP1 show impaired lipid metabolism as well as reduced spine density, diminished synaptic functions and neuroinflammation. Similar observations are made in are APOE4 targeted replacement (APOE4-TR) compared to APOE3-TR mice. We sought to elucidate apoE-LRP1 interaction in primary rat hippocampal and cortical neuron cultures in a apoE genotype dependent manner. ApoE lipid particles resembling astrocyte secreted lipoproteins were prepared, purified and used for the study. Neuronal LRP1 was detected using MMMM antibody in control and apoE treated cultures. Our data indicate genotype and lipid dependent interaction of apoE with LRP1. In control neurons apoE was predominantly located in juxtanuclear compartments with some punctate distribution in processes. Incubation of apoE lipid particles with neurons caused redistribution of juxtanuclear LRP1 to cell surface. ApoE3 lipid particles affected neuronal LRP1 distribution more efficiently compared to apoE4 lipid particles. Whereas lipid free recombinant apoE3 or apoE4 were unable to induce LRP1 redistribution. Increased endocytosis and recycling of LRP1 may be responsible for LRP1 retention at cell surface in presence of apoE3 lipid particles, whereas impaired interaction of apoE4 with LRP1 might contribute to increased AD risk. We are currently investigating apoE genotype dependent interaction between apoE-LRP1 in APOE-TR mice.

Disclosures: I.Y. Tamboli: None. **G. Rebeck:** None.

Nanosymposium

579. Risk Factors for Neurodegenerative Diseases

Location: 150B

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 579.03

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

Support: NIH AG043503

NIH T32-AG000255

NIH AG017586

NIH NS044266

NIH AG032953

NIH AG010124

Wyncote Foundation

Title: A multimodal network associated with asymptomatic frontotemporal degeneration

Authors: *C. MCMILLAN[1], K. RASCOVSKY[2], E. WOOD[2], A. CHEN-PLOTKIN[2], B. AVANTS[2], P. COOK[2], J. POWERS[2], C. OLM[2], L. BAEHR[2], J. GEE[2], V. M. LEE[2], J. Q. TROJANOWKSI[2], V. VAN DEERLIN[2], M. GROSSMAN[2];
[1]Neurol., Univ. of Pennsylvania, PHILADELPHIA, PA; [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: There is increasing evidence for an asymptomatic phase of frontotemporal lobar degeneration (FTLD), but the basis for defining this has proven elusive. As therapeutic disease-modifying agents emerge for clinical trials, it is critical to identify preclinical markers of progressive neurodegeneration to track disease and monitor treatment response. This study aims to identify a network of grey matter (GM) and white matter (WM) regions that is sensitive and specific for asymptomatic GRN mutation carriers (aGRN+), and to evaluate the association between plasma progranulin levels and neuroanatomic changes. In this study we use cross-validation to train a GRN-associated network in symptomatic individuals with FTLD and then validate this network in aGRN individuals and relate it to progranulin plasma levels. We recruited 13 GRN+ FTLD patients and 32 demographically-comparable healthy controls for our training cohort. Related at-risk family members including 12 aGRN+ and 14 aGRN- individuals were recruited for our asymptomatic validation analyses. We identified data-driven volumes-of-interest (VOI) using Eigenanatomy for T1-weighted MRI measures of GM, diffusion tensor imaging (DTI) measures of WM, and a multimodal combination of GM and WM. A logistic regression classifier was used to identify sensitive and specific VOIs in symptomatic GRN+ patients relative to demographically-comparable, GRN- healthy controls [AUC=1.0; $p<0.001$]. This revealed left anterior and orbital frontal, right superior temporal, and left ventromedial prefrontal cortex as sensitive and specific VOIs. The most stable WM VOIs in the training regression included genu and anterior corpus callosum, splenium and parieto-occipital corpus callosum, bilateral inferior longitudinal fasiculus, and bilateral corona radiata including corticospinal tract. Our validation analysis revealed that the multimodal network that included GM and WM data achieved the highest accuracy (AUC=0.881; $p=0.001$) with 83% sensitivity and 93% specificity. GM analysis alone was also accurate (AUC=0.839; $p=0.003$) with 75%

sensitivity and 93% specificity. While less accurate than the GM analysis, the WM analysis still achieved an AUC=0.810 (p=0.007) with 75% sensitivity and 86% specificity. The neuroimaging-based predicted probability of being aGRN+ correlated significantly with progranulin plasma levels in aGRN individuals [r=0.47; p=0.03], but not age [p>0.1]. Together, we conclude that a multimodal network is compromised in GRN and that neuroimaging provides a candidate approach for monitoring the natural history of disease in asymptomatic GRN carriers.

Disclosures: C. McMillan: None. **K. Rascovsky:** None. **E. Wood:** None. **A. Chen-Plotkin:** None. **B. Avants:** None. **P. Cook:** None. **J. Powers:** None. **C. Olm:** None. **L. Baehr:** None. **J. Gee:** None. **V.M. Lee:** None. **J.Q. Trojanowksi:** None. **V. Van Deerlin:** None. **M. Grossman:** None.

Nanosymposium

579. Risk Factors for Neurodegenerative Diseases

Location: 150B

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 579.04

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

Support: William Beaumont Hospital Dept. of Radiation Oncology

Michigan Head and Spine Institute.

Title: External radiation is associated with reduction of aβ plaque burden and hippocampal synaptophysin staining in a murine model of alzheimer's disease

Authors: *D. B. MICHAEL[1], A. HANNA[2], R. HUNT[3], A. MARTINEZ[4], M. MADDENS[2], G. WILSON[2], J. FONTANESI[4], B. MARPLES[2];
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Abstract: Alzheimer’s Disease (AD) represents the most frequent form of dementia and is characterized by brain extra cellular beta-amyloid (Aβ) plaques and Tau neurofibrillary tangles. A progressive degradation in episodic memory is the clinical hallmark of AD. Our group has previously reported radiation therapy (RT), in doses used to cure children of acute lymphocytic leukemia, results in up to an 80% reduction in Aβ plaques in a hemibrain irradiated mouse

model. Last year we reported whole brain RT results in improved Morris Water Maze performance in the same model. This study tests the hypothesis that whole brain RT leads to reduction in Aβ plaque and hippocampal synaptophysin staining in a murine AD model. 16 month old APPswe, PSEN1dE9)85Dbo/J mice were randomized into RT (n=6) or no-RT (n=4) groups. Animals were maintained in accordance with SFN animal housing standards. After behavioral testing was carried out over the following 8 weeks, the animals were sacrificed, the brains rapidly dissected and frozen. The brains were then processed for Aβ and synaptophysin Immunohistochemistry. Images were processed using the Dinfiniens© image processing software. Plaque counts and synaptophysin staining were compared using Student's T test. Aβ plaque counts were reduced in the RT v. nRT brains (mean 3470 v. 5804 p=0.05). Results of synaptophysin dentate, CA1, CA2, and CA3 staining showed a reduction in staining values in RT v.nRT animals. This reduction in CA1 was statistically significant (p=0.0331). These data suggest that radiation therapy may present a new treatment strategy in AD patients by reducing . Aβ plaque burden and altering hippocampal synaptic plasticity.

Disclosures: D.B. Michael: None. **A. Hanna:** None. **R. Hunt:** None. **A. Martinez:** None. **M. Maddens:** None. **G. Wilson:** None. **J. Fontanesi:** None. **B. Marples:** None.

Nanosymposium

579. Risk Factors for Neurodegenerative Diseases

Location: 150B

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 579.05

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

Support: VA CDA 2010-2015

AA NIRP 2014--304720

NIA 2P50AG005138-28

Title: The role of apoe4-induced phospholipid dysregulation in Alzheimer disease pathogenesis

Authors: *D. CAI[1], L. ZHU[1], G. ELDER[1], S. GANDY[1], C. CARDOZO[2], N. ROBAKIS[3]; [1]Neurol., [2]Res. & Develop., James J Peters VA Med. Center/Mount Sinai Sch. of Med., Bronx, NY; [3]Psychiatry, Mount Sinai Sch. of Med., New York, NY

Abstract: The ApoE4 genotype is the strongest risk factor for developing Alzheimer's Disease (AD). However, the mechanisms that underlie the link between ApoE4 genotype and AD are not

well understood. We have found that the levels of PIP_2 are reduced in postmortem human brain tissues of ApoE4 carriers, in the brain of ApoE4 homozygous knockin (KI) mice, and in primary neurons expressing ApoE4 alleles, if compared to ApoE3 counterparts. The expression of synaptojanin 1 (synj1) that dephosphorylates PIP_2 reducing its levels, is elevated in ApoE4 brains. Genetic reduction of synj1 in ApoE4 KI mouse models can restore PIP_2 levelsc omparable to those in ApoE3 mouse brains, and rescue AD-related cognitive deficits in ApoE4 KI mice as determined by Novel Object Recognition (NOR) and Fear Conditioning (FC) studies. Together, our findings suggest that ApoE genotype is a critical determinant of brain phospholipid homeostasis and that the ApoE4 isoform is dysfunctional in this process (increased synj1 expression and reduced PIP_2 levels). These ApoE4-induced in the cascade of aberrant molecular events may lead to long-term neurodegenerative process and AD development. Our studies may uncover new therapeutic options for the treatment of AD targeting at ApoE4 pathogenic nature.

Disclosures: D. Cai: None. **L. Zhu:** None. **G. Elder:** None. **S. Gandy:** None. **C. Cardozo:** None. **N. Robakis:** None.

Nanosymposium

579. Risk Factors for Neurodegenerative Diseases

Location: 150B

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 579.06

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

Support: Helmholz Association

EU project Memories

Title: The pro-neurotrophin receptor sortilin is a major neuronal apoE receptor for catabolism of amyloid-β peptide in the brain

Authors: *A.-S. CARLO[1], C. GUSTAFSEN[3], G. MASTROBUONI[2], S. KEMPA[2], C. M. PETERSEN[3], T. E. WILLNOW[1];
[1]Mol. Cardiovasc. Res., [2]BIMSB, MDC, Berlin, Germany; [3]MIND, Aarhus, Denmark

Abstract: Sortilin is a member of the VPS10P domain receptor gene family, a class of sorting and signalling receptors. In the nervous system, sortilin plays a central role in control of neuronal survival by transmitting proneurotrophin-dependent death signals and by controlling the catabolism of progranulin, an etiologic agent in frontotemporal lobar degeneration. However,

sortilin has also been shown to act as intracellular sorting receptor for apolipoprotein (apo)B100 in hepatocytes and to modulate the release of nascent apoB100-containing lipoproteins from the liver. This observation suggests that pathways in neuronal viability and function and in cellular lipoprotein metabolism may converge on this receptor. In this study, we explored the relevance of sortilin for neuronal actions of apoE, the main apolipoprotein to deliver lipids to neurons and major risk factor for sporadic Alzheimer disease (AD). To address the consequences of impaired sortilin activity for AD-related processes in vivo, we crossed mice with targeted sortilin gene disruption with murine models of AD (PDAPP, 5xFAD lines). Sortilin-deficient mice showed a robust increase in brain apoE levels. Also, concentrations of Aβ in the brain were elevated as compared to control animals. As the levels of soluble APP products or of Aβ-degrading enzymes were not altered in sortilin-deficient mice, these findings suggested an impairment of apoE-dependent clearance pathways for Aβ in animals lacking this receptor. This hypothesis was confirmed by documenting the ability of sortilin to bind all apoE isoforms with high affinity and to mediate cellular uptake and catabolism of apoE and apoE/Aβ complexes in established cell lines. Also, primary neurons lacking sortilin exhibited significantly impaired uptake of apoE/Aβ complexes despite proper expression of other apoE receptors. In spite of higher than normal brain apoE levels, sortilin-deficient animals displayed anomalies in brain lipid metabolism (such as accumulation of sulfatides) seen in apoE-deficient mice, indicating functional deficiency in cellular apoE uptake pathways in these animals. Taken together, our findings identified sortilin as an essential neuronal pathway for clearance of apoE-containing lipoproteins in vivo and the relevance of this pathway for amyloidogenic processes. Currently, we are investigating the physiological relevance of this sortilin-dependent lipid transport machinery for neuronal lipid homeostasis.

Disclosures: A. Carlo: None. **C. Gustafsen:** None. **G. Mastrobuoni:** None. **S. Kempa:** None. **C.M. Petersen:** None. **T.E. Willnow:** None.

Nanosymposium

579. Risk Factors for Neurodegenerative Diseases

Location: 150B

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 579.07

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

Title: Multi-omic analysis of apoE isoform effects in an AD-vulnerable brain region

Authors: *T. NURIEL[1], L. LIU[1], R. CHAN[1], A. DILLMAN[2], Q. CHEN[3], V. DROUET[1], G. DI PAOLO[1], M. COOKSON[2], S. S. GROSS[3], K. DUFF[1];
[1]Columbia Univ. Med. Ctr., New York, NY; [2]Natl. Inst. on Aging, Bethesda, MD; [3]Weill Cornell Med. Col., New York, NY

Abstract: Possession of the ε4 allele of apolipoprotein E (APOE) is a major genetic risk factor for Alzheimer's disease (AD). The prevailing hypothesis is that the accelerated AD pathogenesis observed in APOE ε4 carriers is mediated by the decreased ability of the apoE4 protein to clear Aβ from the brain parenchyma. However, possession of the APOE ε4 allele also results in a number of other neurological deficits unrelated to Aβ clearance, such as decreased dendritic arborization and spine formation, thinner entorhinal cortex layers and poorer outcomes after traumatic brain injury and stroke, suggesting that there may be other mechanisms involved in this process. In order to gain a more comprehensive understanding of the effects of alternative apoE4 isoform expression in the brain and its role in the pathogenesis of AD and other age-related neurological illnesses, "omics" technology was utilized to identify small-molecules, lipids and RNA whose levels are significantly altered in association with alternative apoE isoform expression in an AD-vulnerable vs. an AD-resistant brain region from mice. To accomplish this, a novel 3-in-1 extraction method was utilized to extract lipids, small-molecules and proteins from entorhinal cortex and primary visual cortex tissues obtained from pathologically normal 14-month old apoE targeted replacement (TR) mice expressing either apoE3/3, E3/4 or E4/4. Additionally, trizol extraction was utilized to purify RNA from equivalent tissues dissected from the opposite brain hemisphere of these mice. The levels of the small-molecules, lipids and RNA extracted from these tissues were then measured using untargeted metabolomics, targeted lipidomics and RNA-sequencing, respectively. These studies have uncovered significant changes in small-molecules, lipids, and RNA involved in energy metabolism, the endosomal/lysosomal system, synaptic function and other important cellular processes. Together, this data points to new mechanisms that may be responsible for the increased incidence of AD among APOE ε4 carriers, which may potentially lead to the development of novel therapeutic strategies.

Disclosures: T. Nuriel: None. **K. Duff:** None. **L. Liu:** None. **V. Drouet:** None. **R. Chan:** None. **G. Di Paolo:** None. **S.S. Gross:** None. **Q. Chen:** None. **M. Cookson:** None. **A. Dillman:** None.

Nanosymposium

579. Risk Factors for Neurodegenerative Diseases

Location: 150B

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 579.08

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

Support: Alzheimer's Association NIRG-10-173988

Pine Family Foundation

NIH/NIA P01 AG02250

Doctoral Bridge Funding, UNTHSC

Title: Differential responses to antioxidant and exercise interventions in male and female GFAP-ApoE3 and -ApoE4 mice

Authors: *K. CHAUDHARI, J. M. WONG, P. H. VANN, N. SUMIEN;
Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX

Abstract: The ε4 allele of apolipoprotein E (ApoE) is a well-established genetic risk factor for development of late-onset Alzheimer's disease (AD). To prevent or delay appearance of brain dysfunction, a healthy lifestyle, such as exercising and eating antioxidants, is often recommended. Physical activity has been shown to have an allele-specific beneficial effect on cognition in humans and rodents. Antioxidant therapy is suggested to improve brain function especially in ε4 carriers. Health conscious individuals are likely to combine exercise with antioxidant intake to increase benefits; however recent studies have indicated the potential for a negative interaction of these two factors. Our study aimed at determining the nature of the interaction between exercise and antioxidants on functional outcomes in a model of increased AD risk, and whether sex also influenced the outcomes. Male and female mice (12months), expressing human ApoE3 or E4, were assigned to one of the treatments: sedentary/control diet (SedCon), sedentary /antioxidant-rich diet (Vitamins E and C; SedEC), exercise/control diet (ExCon), exercise/ antioxidant-rich diet (ExEC). The treatments were given for 8 weeks prior and maintained for another 8 weeks during behavioral tests for balance and coordination, spatial learning and memory, and cognitive flexibility. In the coordinated running test, the E3 mice performed better than the E4 mice, and a significant improvement was observed with the ExEC treatment in males E3. In a test for spatial learning and memory, only GFAP- ApoE4 mice exhibited an improved performance with the treatments, especially with the combination (ExEC). In the active avoidance task, the initial learning was improved by the combination of exercise and antioxidants only in females regardless of their genotype. All treatments improved the cognitive flexibility of the females ApoE3 but not ApoE4. In males, only the combination of the two treatments improved the performance of the ApoE3 mice but not the ApoE 4 ones. Hence, sex and genotype may be critical determinants in the functional outcomes of interventions such as exercise and antioxidants. This data provides strong science-based evidence and a strong framework for the development of clinically-useful strategies to improve motor and

cognitive function, and further develop individualized assessment and treatment based on sex and other factors such as genotype.

Disclosures: **K. Chaudhari:** None. **J.M. Wong:** None. **P.H. Vann:** None. **N. Sumien:** None.

Nanosymposium

579. Risk Factors for Neurodegenerative Diseases

Location: 150B

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 579.09

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

Support: NS073899

Title: Epigenetic regulation of FKBP5 in aging and disease

Authors: ***L. J. BLAIR**[1], J. J. SABBAGH[1], T. KLENGEL[2], E. BINDER[2], C. A. DICKEY[1]; [1]Mol. Med., Univ. of South Florida, Tampa, FL; [2]Emory Univ. Sch. of Med., Munich, Germany

Abstract: Single nucleotide polymorphisms (SNPs) in the FKBP5 gene interact with trauma and stress to promote many psychiatric diseases. Combined with stress exposure, FKBP5 SNPs in humans increase the risk of PTSD, major depressive disorder, and suicide. These SNPs boost the intrinsic levels of FKBP5 through a process involving FKBP5 demethylation in non-coding regions, which allows for even higher levels of FKBP5 protein to be made following stress. We have now found that methylation of the FKBP5 gene is also decreased in the aging brain and even more so in the Alzheimer's disease (AD) brain, leading to increased expression of FKBP5. We find that increases in FKBP5 levels in brain can cause both accumulation of the microtubule associated protein tau that is suspected to AD pathogenesis, and altered glucocorticoid receptor (GR) function that is linked to a number of psychological disorders. In an effort to model the epigenetic regulation of FKBP5 in mice for future drug development and mechanistic studies, we generated the first tet-regulatable FKBP5 over-expressing mouse model using targeted insertion at the Rosa26 locus. These mice over-express a single copy of the FKBP5 gene and we found that this was sufficient to reduce methylation of the endogenous fkbp5 gene, modeling the effects of SNPs in humans. Further characterization of this model will help to elucidate the molecular mechanisms of FKBP5 gene regulation, which could lead to important therapeutic approaches in the future for both Alzheimer's disease and psychopathologies.

Disclosures: **L.J. Blair:** None. **J.J. Sabbagh:** None. **T. Klengel:** None. **E. Binder:** None. **C.A. Dickey:** None.

Nanosymposium

580. Parkinson's Disease: Mechanisms and Circuits

Location: 146C

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 580.01

Topic: C.03. Parkinson's Disease

Title: The nature of the brain dopamine storage defect in Parkinson's disease: A study in isolated synaptic vesicles of human caudate and putamen

Authors: *C. PIFL[1], A. RAJPUT[2], H. REITHER[1], J. BLESA[3], C. CAVADA[4], J. A. OBESO[3], A. H. RAJPUT[2], O. HORNYKIEWICZ[1];
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Abstract: A half century after the discovery of the profound nigrostriatal dopamine deficit in Parkinson's disease (PD) brain, the cause of the degeneration of the dopamine neurons is still unknown. Intraneuronally, dopamine is largely confined to synaptic vesicles where it is protected from metabolic breakdown by a low pH and absence of metabolic enzymes. In the cytoplasm, however, the free dopamine can give rise, especially via the monoamine oxidase reaction, to formation of cytotoxic free radicals as well as react with a number of cellular components, including alpha-synuclein, an ubiquitous cellular protein strongly implicated in the pathogenesis of PD. Normally, the cytoplasmic concentration of dopamine is kept at a minimum by the continuous activity of the vesicular monoamine transporter (VMAT)2. Accordingly, defects in the handling of cytosolic dopamine by the VMAT2, or in the vesicular storage of dopamine, including increased dopamine leakeage into the cytoplasm, have variously been hypothesized to lead to increased levels of dopamine-generated oxy radicals ultimately resulting in degeneration of the dopamine neurons in PD. Here, we isolated for the first time, dopamine storage vesicles from the striatum of 6 autopsied brains of PD patients and 4 controls, and directly measured several indices of dopamine storage. Vesicular uptake of [3H]dopamine and binding of the VMAT2 selective label [3H]dihydrotetrabenazine were profoundly reduced in PD by 87-90%

and 71-80%, respectively. This was primarily due to the loss of dopamine nerve terminals but the parallel determination of both parameters in each preparation revealed that transport per monoamine uptake site was also profoundly reduced in PD; after correction for VMAT2 in serotonin terminals (presumed to be unchanged) the reduction of transport in dopamine vesicles proper was by 56% in caudate and by 90% in putamen. This was not the case after a similar degree of nigrostriatal neurodegeneration induced by MPTP in Macaca fascicularis (seven MPTP and eight controls) and seems to be a PD specific finding. Efflux studies and determination of the acidification in the vesicular preparations suggest an injury of the uptake by the VMAT2 in PD which might impair sequestration of reactive dopamine from the cytosol of dopaminergic nerve terminals and contribute to their degeneration. Thus, by directly identifying the vesicular pathophysiologic mechanism that by its nature would promote increased cytosolic dopamine levels, our results strongly support the notion of the role of dopamine cytotoxicity in the degeneration of the nigrostriatal dopamine neurons in PD.

Disclosures: C. Pifl: None. **A. Rajput:** None. **H. Reither:** None. **J. Blesa:** None. **C. Cavada:** None. **J.A. Obeso:** None. **A.H. Rajput:** None. **O. Hornykiewicz:** None.

Nanosymposium

580. Parkinson's Disease: Mechanisms and Circuits

Location: 146C

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 580.02

Topic: C.03. Parkinson’s Disease

Support: K23NS060949

Parkinson's Disease Foundation

T32AG000269-15

Title: Brainstem volume as an imaging biomarker of Parkinson's Disease

Authors: *C. D. SCHROEDER, G. T. STEBBINS, J. G. GOLDMAN;
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Abstract: Objective: To investigate regional brainstem atrophy on neuroimaging in Parkinson’s Disease. **Background:** The neuropathology of Parkinson’s disease (PD) is hypothesized to have early changes in the medulla oblongata and progress to the substantia nigra, midbrain, and eventually the neocortex according to Braak’s staging. Structural magnetic resonance imaging

(MRI) provides an opportunity to examine differences in the brainstem volume values between PD patients and age-matched, healthy controls. Brainstem atrophy detected on MRI scans could potentially be used as a biomarker of PD progression. **Methods:** 101 clinically diagnosed PD patients and 24 age-matched, healthy controls underwent clinical evaluations and MRI brain scans (1.5T GE Signa, T1-weighted sequences, MPRAGE or IR-FSPGR). Participants were recruited as part of a prospective PD imaging study (JGG). Whole brain voxel-based morphometry analyses were conducted using SPM8. Images were smoothed with a 6mm kernel. Regions of interest for midbrain, pons, and medulla gray matter volumes were extracted from modulated non-linear scans using the Wake Forest University Pickatlas to identify regions of interest. Ratios were created by dividing midbrain volume by pons volume (midbrain:pons ratio) and pons volume by medulla volume (pons:medulla ratio). Gray matter volume and volume-ratio differences between the PD and control groups were examined using a MANCOVA, covarying for scan sequence (MPRAGE vs. IR-FSPGR) and gender with significance set at $p<0.05$. **Results:** The PD and healthy control subjects did not differ significantly in age (mean [SD] PD 73.41 [6.31] years, Control 71.75 [6.07] years). The PD group had a mean PD duration of 10.48 [4.62] years. The PD group exhibited significant voxel-wise differences with decreased gray matter volume in the midbrain (mean [SD] PD 0.16 [0.03] Control 0.22 [0.04], $p<0.0005$) and the pons (mean [SD] PD 0.057 [0.012], Control 0.059 [0.010], $p=0.031$). The midbrain:pons ratio was significantly reduced in the PD group as well (mean [SD] PD 2.79 [0.55], Control 3.78 [0.76], $p<0.0005$). Only midbrain:pons volume was correlated with PD duration $r=-0.259$ ($p=0.009$). There were no significant differences found in the medulla or the pons:medulla ratio. **Conclusion:** There is a pattern of gray matter differences in the brainstem between PD and control groups, specifically with atrophy in the midbrain. This finding supports Braak's neuropathological staging of PD-related neurodegeneration in this area.

Disclosures: C.D. Schroeder: None. **G.T. Stebbins:** None. **J.G. Goldman:** None.

Nanosymposium

580. Parkinson's Disease: Mechanisms and Circuits

Location: 146C

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 580.03

Topic: C.03. Parkinson's Disease

Support: CIHR

Title: Substantia nigra diffusion parameters in newly diagnosed parkinson patients: should fractional anisotropy be considered a biomarker?

Authors: *T. ROLHEISER[1], K. P. GOOD[2], R. LESLIE[3], J. FISK[4], G. PHILLIPS[5], R. MCKELVEY[5], K. ROCKWOOD[6], M. WOJTOWICZ[7], O. THEOU[6], D. LEWIS[8], M. ARMSTRONG[8], R. GAN[8], R. STERNICZUK[9], P. CHIASSON[10], M. N. KHAN[11], C. MACKNIGHT[6], K. SCHOFFER[6], M. SCHMIDT[11], A. NEWMAN[2], B. RUSAK[2], H. ROBERTSON[12];
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Abstract: Objective: We examined whether Diffusion Tensor Imaging (DTI) values of the substantia nigra differentiated newly diagnosed Parkinson Disease (PD) patients from matched healthy control subjects. **Background:** Interventions that halt or reverse the progression of PD remain an essential unmet need. Current PD therapies are only applied after clinical diagnosis, when damage may be irreparable. Preclinical PD precedes diagnosis by as much as 4-6 years and involves pathological changes to several subcortical brain structures. Past work using DTI suggests a strong link between the progression of PD and a decrease in fractional fnisotropy (FA) in the substantia nigra. We hypothesized that diffusion measures of the substantia nigra would be markedly decreased in a cohort of newly-diagnosed PD patients, despite the early stage of the clinical diagnosis. **Methods:** Fifteen newly diagnosed PD patients and a matched control group completed behavioral testing, olfactory testing and MRI scanning. DTI analysis of the SN took place in two parts: a whole-brain tract based spatial statistics analysis (TBSS) as well as a region of interest (ROI) analysis of the SN. The ROI analysis was completed by hand tracing the bilateral SN on a V1 color map. **Results:** The ROI analysis of the SN revealed only a non-significant trend towards a decrease in FA for the PD cohort. The whole brain TBSS analysis revealed a small decrease in FA in the left corticospinal tract, with no incursions beyond the central white matter of the internal capsule. **Conclusions:** The preclinical antecedents of PD are hypothesized to involve many subcortical structures beyond the SN, with dopaminergic cell death being among the final stages of disease progression. In this present study we found that in newly diagnosed patients, FA of the SN is not a sensitive marker of disease progression. Complementary analyses are ongoing to determine whether other ROIs might confer a larger effect size during this stage of the disease.

Disclosures: T. Rolheiser: None. **K.P. Good:** None. **R. Leslie:** None. **J. Fisk:** None. **G. Phillips:** None. **R. McKelvey:** None. **K. Rockwood:** None. **M. Wojtowicz:** None. **O. Theou:** None. **D. Lewis:** None. **M. Armstrong:** None. **R. Gan:** None. **R. Sterniczuk:** None. **P. Chiasson:** None. **M.N. Khan:** None. **C. MacKnight:** None. **K. Schoffer:** None. **M. Schmidt:** None. **A. Newman:** None. **B. Rusak:** None. **H. Robertson:** None.

Nanosymposium

580. Parkinson's Disease: Mechanisms and Circuits

Location: 146C

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 580.04

Topic: C.03. Parkinson's Disease

Support: ANR-2010-1416

Association France Parkinson

CNRS

AMU

Title: Striatal cholinergic interneurons and motor symptoms of Parkinson's disease

Authors: *M. AMALRIC[1,3], M. LIBERGE[2], S. ZTAOU[2], N. MAURICE[3], F. JAOUEN[3], L. KERKERIAN-LEGOFF[3], C. BEURRIER[3];
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Abstract: Disturbance in the central muscarinic cholinergic system has been implicated in several neurodegenerative pathology including Parkinson's disease (PD) and Alzheimer's disease. Anticholinergic (ACh) drugs were the first widely accepted drugs in PD. Their precise mechanism of action is still not clear, although it is believed that they work by correcting the disequilibria between striatal dopamine and acetylcholine activity. In this study, we examine the involvement of striatal ACh interneurons in the control of motor function by manipulation of cholinergic activity with use of pharmacologic and optogenetic approaches. Selective muscarinic receptor antagonists (telenzepine and tropicamide, M1 and M4 receptor antagonist, respectively), systemically administered in the 6-OHDA model of PD decreased postural asymmetry and turning bias in the cylinder test and cross maze tests. To further investigate the involvement of dorsal striatal ACh interneurons in these effects, we performed intrastriatal injection of M1 and M4 receptor antagonist and selectively manipulated cholinergic activity in transgenic mice expressing Cre-recombinase under the choline acetyltransferase promoter. Cre-inducible adeno-associated virus vector carrying the gene encoding channelrhodopsin (ChR2) or halorhodopsin (eNpHR) were specifically expressed in striatal ACh interneurons. Electrophysiological recordings of ACh interneurons in vitro in striatal slice and in vivo confirmed that under laser

illumination both opsins were functional: ChR2 drove spike activity while eNpHR silenced firing. Optogenetic inhibition of ACh interneurons in 6-OHDA lesioned mice reduced the motor symptoms, while their activation had no effect. There was no effect of laser illumination in control mice. The present investigation indicates that cholinergic modulation in the dorsal striatal circuit plays a pivotal role in the regulation of the motor symptoms in PD and suggest that blocking muscarinic M1 or M4 cholinergic receptors may help in the symptomatic management of parkinsonism.

Disclosures: **M. Amalric:** None. **S. Ztaou:** None. **N. Maurice:** None. **F. Jaouen:** None. **L. Kerkerian-LeGoff:** None. **C. Beurrier:** None. **M. Liberge:** None.

Nanosymposium

580. Parkinson's Disease: Mechanisms and Circuits

Location: 146C

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 580.05

Topic: C.03. Parkinson’s Disease

Support: NIH/NINDS Grant P50NS071669

NIH R01 NS054976 (TW)

NIH/ORIP infrastructure grant to the Yerkes National Primate Research Center P51OD011132

Title: Comparison between short-term beta phase cross-frequency-coupling and beta band power in subthalamic nucleus local field potentials recorded from monkeys with parkinsonism

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Abstract: The progression of parkinsonism can be modeled in primates with repeated small injections of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). In previous studies we examined local field potential signals (LFPs, recorded with macroelectrodes) from the subthalamic nucleus (STN) in three Rhesus monkeys. The animals were rendered parkinsonian by treatment with MPTP (0.2-0.6 mg/kg given subcutaneously, with 21, 26 and 19 injections respectively for monkey A, B and C). We found that parkinsonism is

associated with changes in the coupling between the phase of beta-band oscillations and the amplitude of oscillations in all frequency bands averaged on time scales of at least 2 minutes (cross frequency coupling [CFC], measured by calculating a modulation index, [MI]). We now re-analyzed data from these animals to examine the MI values and LFP signal power over shorter time intervals. We found that the magnitude and variability of the beta-phase MI (calculated on 10s data epochs) were greater in the parkinsonian state than at baseline. Short-time Fourier transform analysis (STFT) revealed that the MI values (based on beta-phase) and spectral power in the beta band did not correlate on the time scale chosen for this analysis, suggesting that beta-phase MI and beta band power may represent different aspects of abnormal signaling in parkinsonism. Beta-phase MI values changed even after the initial injection (when monkeys were still asymptomatic), suggesting that beta-phase MIs are very sensitive to the effects of the neurotoxin. Grant support: This work was supported by grants from the NIH/NINDS (P50NS071669 and R01 NS054976 (TW)) and an NIH/ORIP infrastructure grant to the Yerkes National Primate Research Center (P51OD011132).

Disclosures: T.H. Sanders: None. **A. Devergnas:** None. **M. Clements:** None. **T. Wichmann:** None.

Nanosymposium

580. Parkinson's Disease: Mechanisms and Circuits

Location: 146C

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 580.06

Topic: C.03. Parkinson’s Disease

Support: National Science Council, Taiwan, grant NSC101-2321-B-002-077

National Science Council, Taiwan, grant NSC102-2321-B-002-058

National Health Research Institute, Taiwan, grant NHRI-EX102-10006NI

National Taiwan University Hospital, grant M1466

Title: Cortico-subthalamic transmission is the causative mechanism of parkinsonian hypokinetic movements

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Abstract: Introduction: The symptomatic pathogenesis of Parkinson's disease (PD), the most prevalent hypokinetic movement disorder, has long been ascribed to the imbalances between the direct and indirect pathways in the basal ganglia circuitry. However, several electrophysiological phenomena remain unexplained by the direct pathway-indirect pathway theory, and the functional significance of the relatively neglected cortico-subthalamic pathway has not been established. Materials and Methods: By locally application of glutamate receptor blockers into STN in 6-OHDA lesioned rats, we evaluated locomotor behaviors including moving distances, moving duration, rearing score, and rotational bias. We recorded in vivo electrophysiological profiles in STN including extracellular single-unit recordings and local field potentials, either continuously or evoked by premotor cortex stimulation. We also used Thy1::ChR2 transgenic mice, which expresses channelrhodopsin-2 in cortical pyramidal neuron and its deep brain projections. By implanting fiber optic cannula into STN, we selectively stimulated cortico-subthalamic fibers optogenetically. Results: We found that inhibition of cortico-subthalamic transmission via N-methyl-D-aspartate NMDA receptor (NMDAR) reverses parkinsonian motor deficits without dyskinetic side effects, and rescues the electrophysiological abnormalities including excessive subthalamic bursts, cortico-subthalamic synchronization, and in situ beta synchronization in both motor cortex and STN. Stimulation of the premotor cortex further characterizes that NMDAR-dependent cortico-subthalamic transmission is functionally overexpressed in PD, and directly responsible for stimulation-dependent bursts and time-locked firings in STN, consistent with the firing pattern-based and synchronization-based pathophysiological views, respectively. Moreover, local application of dopaminergic agent into STN, which dose not rescue the dopamine deficiency in striatum, sufficiently reverses parkinsonian motor deficits. Finally, optogenetic activation of cortico-subthalamic pathway alone sufficiently and instantaneously turns a normal mouse into parkinsonian one. Conclusion: In contrast to the classic theory putting most emphasis on the direct and indirect pathways, we found that overexpressed cortico-subthalamic pathway, which essentially requires NMDAR, is the causative mechanism of parkinsonian motor deficits. The novel prospective should significantly contribute to a more rational revisit of the mechanism underlying basal ganglia function and clinical therapeutics for PD.

Disclosures: **M. Pan:** None. **C. Tai:** None. **W. Liu:** None. **W. Lai:** None. **C. Kuo:** None.

Nanosymposium

580. Parkinson's Disease: Mechanisms and Circuits

Location: 146C

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 580.07

Topic: C.03. Parkinson's Disease

Support: Parkinson's UK Innovation Grant K-1206

Title: Glycosylation profiling in plasma of patients with Parkinson's disease reveals putative biomarkers

Authors: K. GOTOVAC[1], O. GORNIK[2], S. TELAROVIC[3], V. MILETIC[3], L. C. GUEDES[4], J. FERREIRA[4], T. F. OUTEIRO[5], G. LAUC[2,6], *F. BOROVECKI[1,3];
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Abstract: Most proteins are known to be glycosylated and glycans play important structural, functional and regulatory roles in various physiological processes. Glycosylation is not the simple addition of glycan decorations, but a carefully orchestrated process resulting in the creation of specific branched glycans that significantly affect protein structure and function. Glycan biomarkers have been identified in a range of diseases, but the knowledge about their possible role in Parkinson's disease (PD) is still limited. In order to ascertain the putative role glycosylation plays in development and progression of PD, we performed the first comprehensive analysis of human plasma glycome in PD patients. Glycosylation analysis was performed in plasma samples of 199 PD patients and 47 control individuals using high performance liquid chromatography (HPLC). Additionally, we compared the glycosylation patterns observed in plasma samples from PD patients with those observed in plasma and CSF of 18 patients with Dementia with Lewy Bodies (DLB) and 5 control samples. The results of the study showed considerable differences in the glycosylation profiles between PD patients and control subjects, with the majority of differential glycan groups showing decreased levels, indicating a possible disruption of glycosylation pathways in PD. More precisely, of the 10 glycan peaks (GPs) showing significantly different levels, 3 exhibited increased and 7 decreased values in plasma of PD patients. The increased glycan groups included the GP8, GP15 and GP35 peaks, while GP2, GP6, GP7, GP9, GP13, GP20 and GP22 exhibited decreased levels. A difference in glycosylation patterns was also observed between male and female probands, which is in line with previous studies in healthy individuals and patients suffering from various diseases. Altogether, we found increased representation of polysialylated and decreased representation of fucosylated N-glycans in the plasma of PD patients, a finding comparable to the results of glycosylation analyses carried out in patients with other neurodegenerative

diseases. The analysis of CSF samples from DLB patients revealed correlation with PD patients in three glycan groups. In total, our findings strongly suggest that changes in glycosylation patterns may serve as the basis for the development of novel biomarkers which could be useful in early detection of individuals at risk of developing PD. Furthermore, glycan levels could also potentially be used to monitor progression of the disease, as well as in the search for putative neuroprotective treatments.

Disclosures: **K. Gotovac:** None. **O. Gornik:** None. **S. Telarovic:** None. **V. Miletic:** None. **L.C. Guedes:** None. **J. Ferreira:** None. **T.F. Outeiro:** None. **G. Lauc:** None. **F. Borovecki:** None.

Nanosymposium

580. Parkinson's Disease: Mechanisms and Circuits

Location: 146C

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 580.08

Topic: C.03. Parkinson’s Disease

Support: MJFF

Title: Alterations in alpha-galactosidase A activity in Parkinson's disease brain

Authors: ***M. P. NELSON**[1], T. E. TSE[1], D. B. O'QUINN[1], D. G. WARNOCK[2], J. J. SHACKA[1,3];
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Abstract: The autophagy-lysosome pathway (ALP) is disrupted in Parkinson’s disease (PD) and may contribute to alpha-synuclein (α-syn)-associated PD pathogenesis. Alpha-Galactosidase A (α-Gal A) is a soluble lysosomal enzyme that is mutated in the lysosomal storage disorder Fabry disease and is in the same lipid hydrolyzing pathway as glucocerebrosidase (GCase). Mutations in GCase are a strong risk factor for PD, and GCase has been shown previously to regulate the metabolism of α-syn. Deficiency in α-Gal A promotes the progressive accumulation of glycosphingolipids in visceral tissues and vascular endothelium. Several neurological problems are also common in Fabry patients, which may result from visceral vascular pathology and/or resident neuropathology. The ALP has been shown to be altered in human Fabry kidney, and Parkinsonism has been documented in a patient with Fabry disease. Also, α-Gal A deficiency has been reported in leukocytes of PD patients, suggesting a correlation between α-Gal A deficiency

and PD. However, whether α-Gal A deficiency promotes CNS alterations in the ALP and its substrate α-syn has not yet been determined. Using a pre-clinical mouse model of Fabry disease, we assessed neuropathology and neurodegeneration with a focus on the ALP and α-syn. Brains were harvested from approximately 2-year-old wild-type or α-Gal A-deficient mice. Brains of α-Gal A-deficient mice exhibited a robust increase in immunoreactivity for microtubule-associated protein light chain-3 (LC3) and lysosome-associated membrane protein-1 (LAMP-1), suggesting accumulation of autophagosomes and increased lysosomal numbers and/or volume, respectively, although ultrastructural analysis of α-Gal A-deficient mouse brain revealed a relative lack of autophagosome accumulation. Preliminary western blot analysis of brain homogenates from younger mice revealed a relative lack of LC3-II compared to wild-type littermate control, further suggesting a potentially novel means of ALP disruption resulting from α-Gal A deficiency. We also observed a dramatic increase in immunoreactivity for a pathologic species of α-syn phosphorylated at serine 129 (p129S-α-syn) in α-Gal A-deficient mouse brain that was also found to co-localize with immunoreactivity for LC3 and ubiquitin. Furthermore, immunoreactivity for p129S-α-syn co-localized with axonal spheroids in α-Gal A deficient mouse brain, a marker of axonal neurodegeneration. Together these results suggest an important role for α-Gal A in regulating normal function of the ALP and the metabolism of α-syn that may be relevant to the onset and progression of PD, providing for new avenues of research into PD pathogenesis.

Disclosures: **M.P. Nelson:** None. **T.E. Tse:** None. **D.B. O'Quinn:** None. **D.G. Warnock:** None. **J.J. Shacka:** None.

Nanosymposium

580. Parkinson's Disease: Mechanisms and Circuits

Location: 146C

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 580.09

Topic: C.03. Parkinson’s Disease

Title: Structural brain connectivity in parkinson’s disease patients with impulse control disorder: Graph-theoretical analysis

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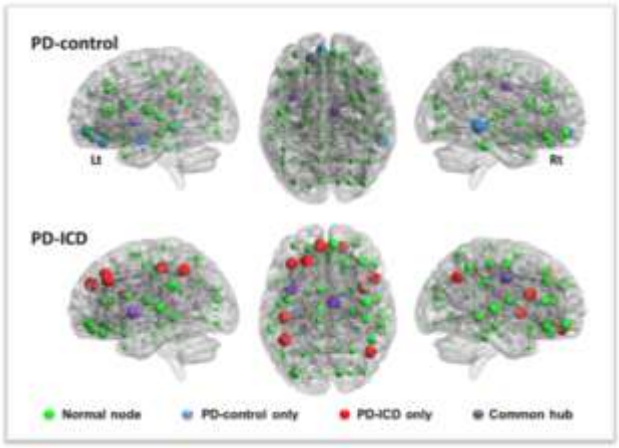
Abstract: Impulse Control disorders (ICD) have long been recognized as an important research topic in Parkinson’s disease patients (PD), however the large-scale brain network in this patients

group is widely unknown. To identify the topological brain network associated with PD who developed ICD, we used structural MRI and voxel-based morphometric (VBM) analysis combined with Graph-Theoretical Analysis (Bullmore and Sporns 2009) In total, 39 patients (25 PD-controls and 14 PD-ICD) underwent structural MRI using a 1.5T scanner (GE Signa). Images were pre-processed using a VBM8 tool-box of the SPM8 with default parameter incorporating the DARTEL. With the correlation matrix of 90 brain sub-regions (region of interest, ROI) from Anatomical Automatic Labeling (AAL), graph-theoretical analysis were conducted using GAT (Hosseini et al. 2012) for analyzing network properties. Global (ie. normalized clustering coefficient, normalized path length, small worldness) and local (ie. nodal degree, nodal betweeness, clustering coefficient) network metrics were calculated at the minimum density as well as across the range of density. In the global network comparison at the minimum density, there were no differences in global network metrics between PD-control and PD-ICD (normalized clustering coefficient: $P = 0.35$, normalized path length: $P = 0.53$, small worldness: $P = 0.34$). Both PD groups showed small-world organization (> 1) however, no significant differences in global network measures between groups were found across the range of densities. Network hub and local network matrix differed between PD-control and PD-ICD in several brain regions (Fig 1), PD-ICD showed higher path traffic in the somatosensory cortex and bilateral frontal areas. The present results showed evidence of altered neural networks in PD-ICD from PD control providing new insights into the neurobiological mechanism of ICD development in PD.



Disclosures: **A. Strafella:** None. **S. Cho:** None. **M. Criaud:** None. **K. Aminian:** None.

Nanosymposium

580. Parkinson's Disease: Mechanisms and Circuits

Location: 146C

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 580.10

Topic: C.03. Parkinson's Disease

Title: Reorganization of structural brain connectivity in parkinson's disease patients with pathological gambling: Generalized structured component analysis

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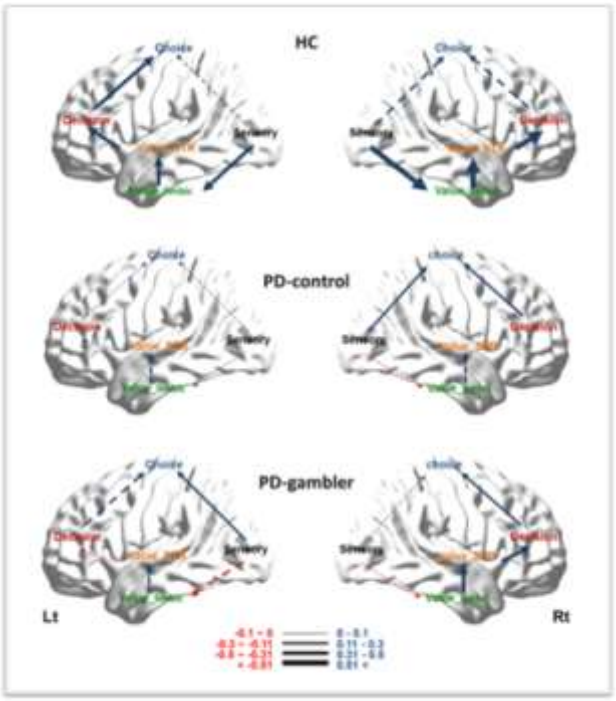
Abstract: Pathological gambling is one of the common impulse control disorders in Parkinson's disease patients (PD) on dopamine replacement therapy. The present study was designed to determine and compare the changes of neural interactions underlying decision making (DM) in PD with gambling problem using structural MRI and voxel-based morphometric (VBM) analysis combined with structural equation modeling (SEM). In total, 28 patients (18 PD-control and 10 PD-gambler) and 18 age-matched healthy controls (HC) underwent structural MRI using a 1.5T scanner (GE Signa). Images were preprocessed using a VBM8 tool-box of the SPM8 with default parameter incorporating the DARTEL. For SEM model specification, brain regions and path directions were defined based on Levy and Glimcher's model (Levy and Glimcher 2012). Generalized structured component analysis (GSCA)(Hwang and Takane 2004), a novel approach to SEM for the analysis of functionally integrated brain connectivity, was applied to a 5-cognitive system (sensory, value-limbic/striatum, decision, and choice) model using each of the corresponding brain regions as latent variable. GSCA showed significant differences in DM neural network among groups in whole model level (overall goodness of fit: $F_{2,299} = 458.0$, $P < 0.001$ in left and $F_{2,299} = 348.4$, $P < 0.001$ in right hemisphere). Most significant differences between PD-control and PG-gambler were limbic/striatum value system to frontal decision pathway, dominantly in the right hemisphere, PD-gambler showed preserved connections while PD-control showed disconnection in this path (Fig1). The present study allowed us to identify a specific change of brain network in PD with gambling problem. Preserved limbic/striatum value system to frontal decision pathway of the right hemisphere in PD-gamblers may modulate weight of reward-related emotional value for output decision which guides the impulsive choice in this patients group.



Disclosures: **S. Cho:** None. **K. Jung:** None. **K. Aminian:** None. **E. Abi-Jaoude:** None. **H. Hwang:** None. **A.P. Strafella:** None.

Nanosymposium

580. Parkinson's Disease: Mechanisms and Circuits

Location: 146C

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 580.11

Topic: C.03. Parkinson’s Disease

Support: Parkinson's Society Canada

CIHR

Title: Aberrant dopamine in the salience network and parahippocampal gyrus contributes to memory impairment in Parkinson’s disease

Authors: *L. CHRISTOPHER[1,2], C. MARRAS[3,2], S. DUFF-CANNING[2], Y. KOSHIMORI[1,2], R. CHEN[3], I. BOILEAU[1], B. SEGURA[1], A. LANG[3], P. RUSJAN[1], S. HOULE[1], A. STRAFELLA[3,2,3];

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Abstract: Background: Patients with Parkinson's disease (PD) and mild cognitive impairment (MCI) often experience memory deficits. Brain networks modulated by dopamine (DA) interact to facilitate memory, and are known to be dysfunctional in PD. Memory deficits could be related to dysfunction executive processing or alternatively dysfunction in the medial temporal lobe. Hypothesis: The objective was to investigate dopaminergic changes in the 1) salience and central executive networks 2) medial temporal lobe 3) associative and limbic striatum in PD patients with memory impairment (amnestic MCI) using PET. We hypothesized that compared to patients with no MCI, patients with non-memory deficits (non-amnestic MCI), and healthy controls, PD patients with amnestic MCI would demonstrate reduced D2 receptor levels in the medial temporal lobe, salience and central executive networks, as well as more severe striatal dopamine depletion in the associative and limbic striatum. Methods: PD amnestic MCI (n=9), PD non-amnestic MCI (n=10), PD with no MCI (n=11) and age-matched healthy controls (HC) (n=14) were recruited. Patients were considered MCI if 2 tests were impaired (≥1.5 std below normative mean) on a neuropsychological test battery. They were split into amnestic (memory impaired) and non-amnestic types using a median split of the composite z-scores for episodic memory. They were scanned with [^{11}C] FLB 457 to measure D2 receptor availability in the cortex, and [11C] DTBZ to measure striatal dopamine denervation. Results: PD patients with memory impairment demonstrated more significant and widespread D2 receptor reductions in the salience network extending from the bilateral insula into the bilateral anterior cingulate cortex (ACC), as well as the right parahippocampal gyrus (PHG) compared to healthy controls. They also had reduced D2 binding in the bilateral insula, left ACC and right PHG comapred to patients with no MCI. They had signfiicant D2 reductions in the right insula and right ACC compared to non-amnestic MCI patients. Furthermore there was a correlation between D2 levels in the right PHG and left insula with composite z-scores for memory in patients with memory impairment. These findings demonstrate the contribution of dopaminergic changes in the salience network and PHG to memory dysfunction in PD.

Disclosures: L. Christopher: None. **C. Marras:** None. **S. Duff-Canning:** None. **Y. Koshimori:** None. **R. Chen:** None. **I. Boileau:** None. **B. Segura:** None. **A. Lang:** None. **P. Rusjan:** None. **A. Strafella:** None. **S. Houle:** None.

Nanosymposium

581. Ischemia: Cellular Mechanisms and Neuroprotection II

Location: 156

Time: Tuesday, November 18, 2014, 1:00 PM - 3:00 PM

Presentation Number: 581.01

Topic: C.08. Ischemia

Support: NIH-NINDS; 2R01NS060896-05 [CA-0051853]

Title: Early brain injury disturbs spontaneous correlated cortical activity in the developing brain

Authors: *S. RANASINGHE[1], G. OR[1], C. M. NIELL[2], V. CHAU[5], P. K. H. WONG[6], H. C. GLASS[3], J. SULLIVAN[3], P. S. MCQUILLEN[4];
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Abstract: Neonatal hypoxic-ischemic (HI) brain injury often results in long-term cognitive and learning impairments in prematurely born human infants. Viable preterm birth typically happens within in a 'pre-critical period' of activity dependent development characterized by the onset of spontaneous and evoked patterned electrical activity that drives the formation of cortical circuits. Reduced background activity on electroencephalogram (EEG) is a sensitive marker of brain injury in human preterm infants that predicts poor neurodevelopmental outcome. We investigated the effects of injury on both general background and specific patterns of cortical activity measured with EEG in a rodent model of very early HI brain injury and in a cohort of human preterm newborns. EEGs were recorded using biparietal, epidural electrodes in rat pups following postnatal day 2 HI or in controls. In human preterm infants, we analyzed EEG recordings obtained in a subset of a larger cohort of newborns studied with early magnetic resonance imaging to identify brain injury. Background activity was analyzed using amplitude integrated EEG (aEEG) that was derived in the standard manner (band pass filter, rectify, time compressed plot). In injured animals, aEEG upper and lower margins are significantly lower following HI compared with control or hypoxia only ($P =/< 0.001$). We also found a significantly decreased aEEG lower margin in injured human newborns compared with controls ($P = 0.003$). EEG recordings were further analyzed to measure interburst intervals (IBI) and to identify bursts of activity in the spindle frequency band (8-30Hz) using an automated analysis. Population analysis of IBI's demonstrates a shift towards longer IBIs in HI injured rat pups ($P < 0.001$) and in injured human neonates ($P = 0.02$) compared to the uninjured controls. In developing rodent cortex, a characteristic activity pattern is the cortical spindle burst that is defined as a field oscillation at frequencies between 8-30 Hz. Cortical spindle bursts may occur as isolated events, or may be coincident with slow frequency events termed slow activity transients (SAT). We found that these spindle-like oscillations were suppressed both in the HI injured rodent brain (I vs C 1.4 vs. 5.8 at P8, $P < 0.05$) and in the injured human brain (I vs C 5.3 vs. 8.1, $P < 0.01$). In the animal model, reduced activity was associated with a spectrum of abnormalities in activity-

dependent development such as delayed expression of glutamate receptor subunits and transporters and reduced dendrite development and spine formation in cortical pyramidal neurons. We are exploring the degree to which restoration of cortical activity can reverse these changes.

Disclosures: S. Ranasinghe: None. **G. Or:** None. **C.M. Niell:** None. **V. Chau:** None. **P.K.H. Wong:** None. **H.C. Glass:** None. **J. Sullivan:** None. **P.S. McQuillen:** None.

Nanosymposium

581. Ischemia: Cellular Mechanisms and Neuroprotection II

Location: 156

Time: Tuesday, November 18, 2014, 1:00 PM - 3:00 PM

Presentation Number: 581.02

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Grant P01 NIA AG014930, Project 1 to RRR

Dr. Miriam and Sheldon G Adelson Medical Research Foundation grant to RRR, and GC

Stanley Medical Research Institute grant to EBH

The Burke Foundation

Goldsmith Foundation

Title: Hydroxamic based histone deacetylase (HDAC) inhibitors can mediate neuroprotection independent of HDAC inhibition

Authors: *S. SLEIMAN[1], D. E. OLSON[2], M. W. BOURASSA[3], S. S. KARUPPAGOUNDER[3], Y.-L. ZHANG[2], J. GALE[2], F. F. WAGNER[2], G. COPPOLA[4], J. PINTO[5], E. HOLSON[2], R. R. RATAN[3];
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Abstract: Histone deacetylase (HDAC) inhibition improves function and extends survival in rodent models of a host of neurological conditions including stroke, and neurodegenerative diseases. Our understanding, however, of the contribution of individual HDAC isoforms to neuronal death is limited. In this study, we utilized selective chemical probes to assess the

individual roles of the class I HDAC isoforms in protecting Mus musculus primary cortical neurons from oxidative death. We demonstrated that the selective HDAC8 inhibitor PCI-34051 is a potent neuroprotective agent and by taking advantage of both pharmacological and genetic tools, we established that HDAC8 is not critically involved in PCI-34051's mechanism of action. We used BRD3811, an inactive ortholog of PCI-34051, and show that despite its inability to inhibit HDAC8, it exhibits robust neuroprotective properties. Furthermore, molecular deletion of HDAC8 proved insufficient to protect neurons from oxidative death, while both PCI-34051 and BRD3811 were able to protect neurons derived from HDAC8 knockout mice. Finally, we designed and synthesized a second, orthogonal negative control compound, BRD5945, that lacks the hydroxamic acid motif and showed that it is not neuroprotective. These results indicate that the protective effects of these hydroxamic acid-containing small molecules are likely unrelated to direct epigenetic regulation via HDAC inhibition, but rather due to their ability to bind metals and inhibit pathological ERK activation. Our results suggest that hydroxamic acid-based HDAC inhibitors may mediate neuroprotection via HDAC-independent mechanisms and affirm the need for careful structure-activity relationship studies when using pharmacological approaches.

Disclosures: S. Sleiman: None. **D.E. Olson:** None. **M.W. Bourassa:** None. **S.S. Karuppagounder:** None. **Y. Zhang:** None. **J. Gale:** None. **F.F. Wagner:** None. **G. Coppola:** None. **J. Pinto:** None. **E. Holson:** None. **R.R. Ratan:** None.

Nanosymposium

581. Ischemia: Cellular Mechanisms and Neuroprotection II

Location: 156

Time: Tuesday, November 18, 2014, 1:00 PM - 3:00 PM

Presentation Number: 581.03

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant NS071056

NY State Spinal Cord Injury Research Program CO19772

Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Burke Medical Research Foundation

Title: Role of acetylation in the repair of DNA double-strand breaks in neurons

Authors: *C. BROCHIER[1,2], R. MEYER[1], G. DENNIS[1], B. LANGLEY[1,2];
[1]Burke/Cornell Med. Res. Inst., White Plains, NY; [2]Brain and Mind Res. Institute, Weill Cornell Med. Col., New York, NY

Abstract: The integrity of the genome is continuously challenged by both endogenous and exogenous DNA damaging agents. Neurons, due to their post-mitotic state, high metabolism, and longevity are particularly prone to the accumulation of DNA lesions. As such, DNA damage is suggested to be a major contributor to both age-associated neurodegenerative diseases and acute neurological injury. A significant body of evidence suggests that histone acetylation, which is regulated by the concerted actions of histone acetyltransferases (HATs) and histone deacetylases (HDACs), plays a central role in the chromatin remodeling that occurs in response to DNA damage. Indeed, chromatin-modifying drugs such as HDAC inhibitors have emerged as attractive therapeutic compounds for neurodegeneration in the last decade. We have recently shown that pharmacological inhibition of HDAC activity could effectively protect neurons against DNA damage-induced apoptosis by disrupting the pro-death transcriptional program of the tumor-suppressor protein p53 (Brochier et al., 2013). However, it is unknown whether HDAC inhibition can promote repair of the DNA lesions in neurons, which is crucial to maintain long-term genomic integrity and neuronal survival. One limitation in understanding DNA repair in neurons, is that most of what is known comes from studies performed in cycling cells or tumors, and might be therefore irrelevant to post-mitotic cells. Our current line of investigation is to determine the molecular mechanisms specific to neuronal DNA repair and the contribution of HDAC activity.

Disclosures: **C. Brochier:** None. **R. Meyer:** None. **G. Dennis:** None. **B. Langley:** None.

Nanosymposium

581. Ischemia: Cellular Mechanisms and Neuroprotection II

Location: 156

Time: Tuesday, November 18, 2014, 1:00 PM - 3:00 PM

Presentation Number: 581.04

Topic: C.08. Ischemia

Support: NSF IOS1121129

Title: Sex differences in the regulation of DNA demethylation following brain injury

Authors: *M. E. WILSON, K. MURRAY, J. WESTBERRY;
Dept Physiol., Univ. of Kentucky, Lexington, KY

Abstract: 17-beta estradiol (E2) protects the cortex from neuronal cell death caused by brain injuries such as middle cerebral artery occlusion (MCAO). This protection is largely dependent on the early presence of estrogen receptor alpha (ERalpha) in the cortex. ERalpha, however, is only transiently expressed in the cortex during neonatal development and is virtually absent in the uninjured adult brain. ERalpha mRNA is re-expressed following MCAO by demethylation of the promoter. To extend these studies, female mice underwent ovariectomy and E2 or oil-vehicle replacement followed by permanent MCAO 1 week later. An additional group included intact males. Brains were removed and snap-frozen at 24 hours following surgery. Two 2mm micro-punches were collected from both sides of the cortex. Punches were used for collection of mRNA or genomic DNA for real time PCR or DNA methylation analysis, respectively. In both female groups, there was an increase in mRNA with a corresponding decrease in DNA methylation of the ERalpha promoter C, but no change in males. There was also a correlative decrease in DNMT1 mRNA expression in females only following injury. Recent studies have shown a role for Tet1, an enzyme that catalyzes the conversion of 5-methylcytosine (5mc) of DNA to 5-hydroxyl-methylcytosine (5hmC) in demethylation of DNA in the brain. Quantitative real time PCR for Tet1 mRNA showed Tet1 mRNA was significantly increased on the injured side of both female groups but not in males. These data are the first to demonstrate a sex difference between changes in methylation of an important gene following MCAO and most importantly, a sexually dimorphic response of Tet1 following injury. Supported by NSF IOS1121129.

Disclosures: **M.E. Wilson:** None. **J. Westberry:** None. **K. Murray:** None.

Nanosymposium

581. Ischemia: Cellular Mechanisms and Neuroprotection II

Location: 156

Time: Tuesday, November 18, 2014, 1:00 PM - 3:00 PM

Presentation Number: 581.05

Topic: C.08. Ischemia

Support: National Natural Science Foundation of China (81230026)

Title: OCT4B-190 exerts neuroprotection after stroke via modulating GSK3β and HDAC6

Authors: ***Y. CHEN**, Z. WU, X. ZHU, X. ZANG, J. JIN, Y. XU;
Neurol., Affiliated Drum Tower Hosp. of Nanjing Univ., Nanjing, China

Abstract: Abstract: OCT4 is a key regulator in maintaining the pluripotency and self-renewal of embryonic stem cells (ESCs). Human OCT4 gene has three mRNA isoforms, termed OCT4A, OCT4B, and OCT4B1. OCT4A mRNA can translate into OCT4A protein. OCT4B mRNA has been recently found to generate three protein isoforms by alternative translation initiation, including the 265-amino-acid protein isoform OCT4B-265, the 190-amino-acid protein isoform OCT4B-190, and the 164-amino-acid protein isoform OCT4B-164. OCT4A is now widely recognized as a transcription factor responsible for the stemness of ESCs, while the biological functions of OCT4B protein isoforms are still not identified. A previous study showed that OCT4B-190 functioned in stress response. In this study, we further investigated biological roles of OCT4B-190 in stroke setting in vivo and in vitro. Using primary neuron cultures, we demonstrated that OCT4B-190 overexpression enhanced neuronal viability after oxygen-glucose deprivation (OGD) treatment, while downregulated stroke-induced histone deacetylase 6 (HDAC6) and glycogen synthase kinase-3β (GSK-3β). Furthermore, it was shown that HDAC6 and GSK3β were co-expressed in the cytosol of neurons, and OCT4 had an effect on interactions between HDAC6 and GSK3β after OGD. Moreover, in male C57BL/6 mice subjected to transit middle cerebral artery occlusion (tMCAO), OCT4B-190 overexpression reduced post-stroke infarct volume and improved neurological functions. These findings suggest that HDAC6 and GSK-3β are involved in pathogenesis of ischemic stroke. OCT4 exerts neuroprotection by inhibition of HDAC6 and GSK-3β.

Disclosures: **Y. Chen:** None. **Z. Wu:** None. **X. Zhu:** None. **X. Zang:** None. **J. Jin:** None. **Y. Xu:** None.

Nanosymposium

581. Ischemia: Cellular Mechanisms and Neuroprotection II

Location: 156

Time: Tuesday, November 18, 2014, 1:00 PM - 3:00 PM

Presentation Number: 581.06

Topic: C.08. Ischemia

Support: Research was supported by RI-INBRE and the NIH National Center for Research Resources (NCRR) 5P20RR016457

Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health under grant R15HD077544

Title: Inter-alpha inhibitor proteins prevent complex auditory processing deficits following neonatal brain injury in rats

Authors: *S. W. THRELKELD[1], M. E. LA RUE[2], C. M. GAUDET[3], Y.-P. LIM[4], B. S. STONESTREET[5];
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Abstract: Neonatal hypoxia-ischemia occurs when brain oxygen and blood flow levels are reduced, leading to injury and subsequent learning and sensory processing impairments. Inter-alpha inhibitor proteins (IAIPs) are serine protease inhibitors found in human plasma and are known to help reduce acute inflammation (Singh et al., 2010). We hypothesized that IAIPs would improve auditory discrimination following neonatal hypoxia-ischemia when subjects were tested as adults (postnatal day 150+). Subjects (HI+vehicle, HI+IAIPs, Sham) were assessed on three auditory processing tasks, which included simple pre-pulse inhibition (sensory motor gating), silent gap detection (temporal processing, 0-100ms) and complex (oddball) tone order discrimination. Auditory processing was comparable for all treatment groups on the pre-pulse inhibition task and the long duration (0-100ms) silent gap detection task. Results revealed significant deficits in complex two-tone discrimination for rats with neonatal HI injury as compared to both sham and HI animals treated with IAIPs. Finally, adult histology revealed significant reductions in cortical and hippocampal volumes for neonatal HI injured rats as compared to shams suggesting regionally specific tissue sparing in IAIP treated subjects. We conclude that treatment with IAIPs following neonatal HI protects against complex auditory processing deficits and regionally specific brain tissue loss seen in untreated neonatal HI injured rats.

Disclosures: S.W. Threlkeld: None. **M.E. La Rue:** None. **C.M. Gaudet:** None. **Y. Lim:** None. **B.S. Stonestreet:** None.

Nanosymposium

581. Ischemia: Cellular Mechanisms and Neuroprotection II

Location: 156

Time: Tuesday, November 18, 2014, 1:00 PM - 3:00 PM

Presentation Number: 581.07

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Molecular hydrogen suppresses apoptosis in neuron and oligodendrocyte caused by neonatal exposure to general anesthetics in mice

Authors: *S. YUFUNE, T. SHIMADA, R. YONAMINE, Y. SATOH, T. KAZAMA;
Dept. of Anesthesiology, Natl. Def. Med., Tokorozawa, Saitama, Japan

Abstract: In animal models, neuronal apoptosis was induced by several anesthetics in the developing brain. Although the mechanisms are controversial, the neurotoxicity may be due to elevated oxidative stress caused by mitochondrial dysfunction. Previously, we reported that hydrogen inhalation as part of the carrier gas mixture during sevoflurane anesthesia can effectively suppress neuronal toxicity in the developing brain (Yonamine et al., Anesthesiology 2013; 118:105-13). In the current study, we sought to investigate whether hydrogen inhalation could effectively protect neuronal and glial cells against toxicity caused by neonatal desflurane exposure. Six-day-old C57BL/6 mice were exposed to 5.6% desflurane for 6 h with or without 1.3% hydrogen as part of the carrier gas mixture. Apoptosis was evaluated by immunohistochemical staining for cleaved caspase-3 (AC-3; marker for apoptosis) and by the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL). Western blot analysis for cleaved poly-(adenosine diphosphate-ribose) polymerase was also performed to examine apoptosis. Furthermore, to evaluate the type of apoptotic cells, we investigated double staining for AC-3 with the cell-type specific markers (NeuN: marker for neurons, glial fibrillary acidic protein (GFAP): marker for astrocytes, or 2',3'-Cyclic-nucleotide 3'-phosphodiesterase (CNPase): marker for oligodendrocytes).Western blot analysis showed that coadministration of 1.3% hydrogen gas significantly reduced the level of neuronal apoptosis compared with desflurane exposure alone. Immunohistochemical analysis showed that the numbers of AC-3+ cells in mice with 1.3% hydrogen were decreased compared with desflurane exposure alone. Double staining showed that AC-3 immunoreactive signals were detected in NeuN+ or CNPase+ cells. On the contrary, no AC-3 immunoreactive signal was detected in GFAP+ cells. Molecular hydrogen suppressed the AC-3 immunoreactive signals in these cells. These results indicate that molecular hydrogen suppresses apoptosis in neuron and oligodendrocyte caused by neonatal exposure to general anesthetics in mice.

Disclosures: S. Yufune: None. **T. Shimada:** None. **R. Yonamine:** None. **Y. Satoh:** None. **T. Kazama:** None.

Nanosymposium

581. Ischemia: Cellular Mechanisms and Neuroprotection II

Location: 156

Time: Tuesday, November 18, 2014, 1:00 PM - 3:00 PM

Presentation Number: 581.08

Topic: C.08. Ischemia

Support: NS44025 (Z.V.)

NS76726 (Z.V.)

Title: The effects of genetic deletion of galectin-3 on microglia activation after acute neonatal focal stroke

Authors: *S. CHIP, F. LI, N. DERUGIN, J. FAUSTINO, Z. VEXLER;
Dept. of Neurology, Univ. California San Francisco, San Francisco, CA

Abstract: Background: Galectin-3 (Gal3) has multiple effects in the extracellular matrix and has been shown to be upregulated in microglia and macrophages in the injured CNS. Expression of Gal3 in microglia has been shown protective following middle cerebral artery occlusion (MCAO) in the adult, but injurious after hypoxia-ischemia in the neonate. Objective: The present study seeks to determine the role of Gal3 in injury after acute neonatal arterial stroke. Methods: Postnatal day 10 (P10) wild-type (WT) and Gal3 knockout (Gal3KO) mice were subjected to 3h MCAO followed by 24h reperfusion. Injury volume was determined in Nissl stained coronal sections. Microglial coverage and cell proliferation were evaluated by Iba1 and 5-bromo-2'-deoxyuridine (BrdU) immunofluorescence, respectively. Cell numbers were analyzed and quantified by Volocity software. Activation of microglial cells was determined by multi-color flow cytometry in homogenates from injured and non-injured cortex pre-incubated with myelin-removal beads. Results: At 24h after MCAO, there was no difference in the volume of injury between Gal3KO (54.6% ± 7.7%) and WT (56.6% ± 3.3%). In the caudate region, an area most prone to injury after acute MCAO, the number of $Iba1^+$ microglia was comparable between WT and Gal3KO (124 ± 28 Vs. 130 ± 24 per 4.3 $x10^{-5}$ ± 9.7 $x10^{-6}$; 4.5$x10^{-5}$ ± 8.3 $x10^{-6}$ μm^3 voxel, respectively). Furthermore, the number of $BrdU^+$ cells in the injured caudate regions of WT and Gal3KO was similar (27± 11 Vs. 25 ± 12 per 9.3 $x10^{-6}$ ± 3.8 $x10^{-6}$; 8.6 $x10^{-6}$ ± 4.1 $x10^{-6}$ μm^3 voxel, respectively). We used multi-color flow cytometry to characterize accumulation of $CD45^+$ and $CD11b^+$ cells in relation to the number of Gal3-expressing and Toll-like receptor 2 (TLR2)-expressing cells. Percent of $CD45^{high}$ cells (invading monocytes and endogenous macrophages) as opposed to $CD45^{low}$/$CD45^{medium}$ (quiescent and activated microglia) was not significantly different between injured regions of WT and Gal3KO mice. Percent of $CD11b^+$ cells (microglia) increased from 17.4 ± 4.4% in contralateral cortex to 26.1 ± 7.3% in injured cortex in WT and from 24.3 ± 2.5% to 45.6 ± 18.4% in KO mice ($p<0.05$ in injured regions; n=5-6/group). A significantly higher number of $CD11b^+$ cells expressed TLR2 in injured cortex of Gal3KO mice than of WT (91.6 ± 8.7% Vs. 61.6 ± 12.3%, respectively; $p<0.05$). Conclusions: Gal3 does not affect injury volume and microglial proliferation but affects accumulation of $CD11b^+$ cells and TLR2 expression early post-injury, suggesting Gal3 involvement in microglia activation.

Disclosures: S. Chip: None. **F. Li:** None. **N. Derugin:** None. **J. Faustino:** None. **Z. Vexler:** None.

Nanosymposium

582. Visual Processing: Faces

Location: 143A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 582.01

Topic: D.04. Vision

Support: ERC Grant facessvep 284025

FNRS Grant FC91608

Title: Intracerebral recording of an objective face detection threshold in the human ventral temporal cortex

Authors: J. LIU-SHUANG[1], J. JONAS[1,2], J. M. ALES[3], A. M. NORCIA[4], L. MAILLARD[2], *B. ROSSION[5];
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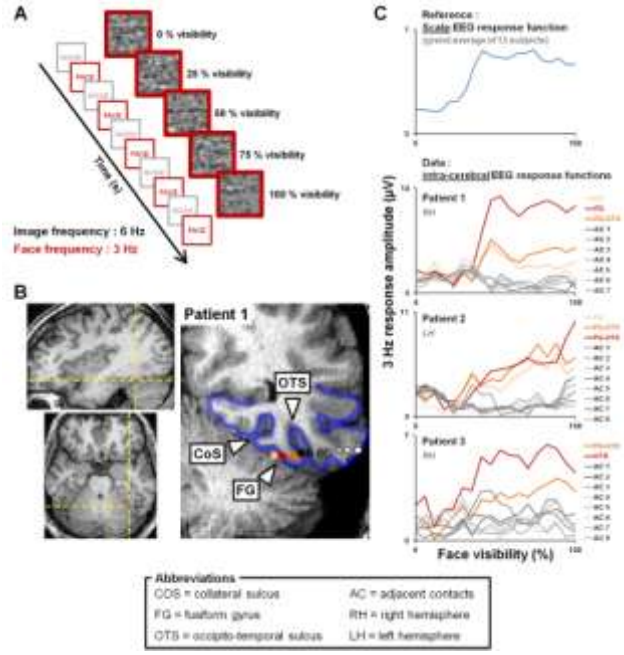
Abstract: Neuroimaging studies have revealed a network of regions in the ventral temporal cortex more responsive to faces than to non-face stimuli, but the precise neural mechanism of face detection in these regions remains unclear. Here we investigated neural face detection responses inside the human brain by means of intracerebral electroencephalography and an original sweep steady-state visual evoked potential (SSVEP) approach (Ales et al., 2012). Three epileptic patients, implanted with depth-electrodes within ventral temporal regions were presented with sequences of face stimuli alternating at a fixed rate with phase-scrambled noise stimuli (6 images/second = 6 Hz). The visibility of the face stimuli was parametrically varied by increasing the phase-coherence of the face so that it gradually emerged over the course of a trial. The power spectrum and contrast were kept equalised between face and noise stimuli throughout the trials. The onset of responses at the exact presentation rate of the face stimuli (3 images/second = 3 Hz) provided an objective marker for face-detection (Figure 1A). In all 3 patients, robust 3 Hz face detection responses were observed only on a limited subset of the ventral occipito-temporal electrode contacts that were among the most face-selective as determined by an independent face-localiser experiment. These contacts were located in the fusiform gyrus and occipito-temporal sulcus (Figure 1B, in red tones) and displayed a non-linear 3 Hz response function that abruptly increased around ≈40% of face visibility followed by a plateau for the next visibility levels (Figure 1C, bottom rows). This response pattern corresponds to the profile that was previously observed on the scalp (Figure 1C, top row). These observations

highlight a face detection mechanism based exclusively on high-level visual properties, located specifically in the fusiform gyrus/occipito-temporal sulcus region of the face perception network. In this region, the face percept emerges through to a step-wise function (i.e. a threshold) rather than a slow accumulation of evidence.



Disclosures: J. Liu-Shuang: None. **B. Rossion:** None. **J. Jonas:** None. **J.M. Ales:** None. **A.M. Norcia:** None. **L. Maillard:** None.

Nanosymposium

582. Visual Processing: Faces

Location: 143A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 582.02

Topic: D.04. Vision

Support: Royal Society Travel for Collaboration grant TG102269

Marie-Curie fellowship 236021

National Science Foundation BCS0923763 and NIMH 54246

National Science Foundation BCS-CAREER-1151805

Wellcome Trust

Title: Ventral visual stream (&EBA) only critical for person perception, not for biological motion perception: Evidence from patients and a model suggestion

Authors: *S. GILAIE-DOTAN;
Inst. of Cognitive Neurosci., UCL, London, United Kingdom

Abstract: Different posterior cortical regions are consistently activated when viewing body movements or static body images (pSTS, FBA, and EBA), yet their distinct functional roles including how they code information remains elusive. Using a paradigm that is sensitive to deficits in biological motion perception (Saygin 2007), we tested five patients with ventral visual lesions and control groups (including n>50 brain damaged patients without ventral cortex damage (Saygin 2007)). We found that ventral visual cortex is not critical for the perception of and sensitivity to biological motion, as evident from the patients' effortless recognition of point light displays and their normal perceptual thresholds. Lesion delineation indicates that EBA or FBA damage does not impair biological motion perception. However, these ventral patients have form perception deficits including three with form and face agnosia that cannot even recognize people from full-body static images. Following these and previous findings I propose a model that outlines the critical functional contributions of pSTS to biological motion perception, and of EBA and FBA to human body perception. More generally, pSTS processes kinematics of self-moving objects. For body movements, part of the representation consists of low resolution static body-in-motion snapshots. Fusiform regions engage in high spatial resolution visual representation of objects, with enhanced representation for self-moving objects due to their varying appearances. The model posits that these representations are experience/familiarity based, explaining the sensitivities to biological motion [human body] in pSTS [FBA], the human biological motion inversion effect (absence of exposure to inverted stimuli leads to absence of representation), and additional findings including in clinical populations. Furthermore, the model provides testable predictions for future research.

Disclosures: S. Gilaie-Dotan: None.

Nanosymposium

582. Visual Processing: Faces

Location: 143A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 582.03

Topic: D.04. Vision

Support: Human Frontier Science Program (HFSP) Long-Term Fellowship

Irma T. Hirschl/Monique Weill-Caulier Trusts Award

Pew Scholar Award in the Biomedical Sciences

McKnight Scholars Award

Title: Resting state networks of the macaque face patch system

Authors: *C. M. SCHWIEDRZIK[1], W. ZARCO[1], S. EVERLING[2,3], W. A. FREIWALD[1]; [1]Lab. of Neural Systems, Rockefeller Univ., New York, NY; [2]Brain and Mind Inst., [3]Robarts Res. Inst., Univ. of Western Ontario, London, ON, Canada

Abstract: Face processing in the macaque monkey occurs in a set of highly specialized brain areas, the "face patches". These areas form a spatially distributed network in which information such as identity, head orientation, and facial expression is extracted. Since face processing is a network phenomenon, it is critical to understand the connectivity within this network in order to understand how face processing comes about. Here, we use resting state functional magnetic resonance imaging (rsfMRI) in six macaque monkeys to noninvasively assess the functional connectivity within and beyond the face patch system at high resolution and with full brain coverage. We find a network of functional connections between faces patches within the superior temporal sulcus (STS) and between the STS and orbitofrontal cortex. Furthermore, we determine the connectivity of each face patch, which includes areas in occipital, parietal, and prefrontal cortex, including premotor areas. Finally, we show that there is significant overlap between the face patch rsfMRI connectivity maps and another large scale network, the default mode network, in the posterior STS and other regions which are involved in social processing in humans. Hence, unraveling the face processing network may not only reveal the larger embedding of this highly specialized network, but may also provide a window into studying the evolution of social skills in primates.

Disclosures: C.M. Schwiedrzik: None. **W. Zarco:** None. **W.A. Freiwald:** None. **S. Everling:** None.

Nanosymposium

582. Visual Processing: Faces

Location: 143A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 582.04

Topic: D.04. Vision

Support: NSF STC award CCF-1231216

Title: Decoding what types of information are in the macaque face patch system

Authors: *E. M. MEYERS[1], M. BORZELLO[2], W. FREIWALD[3], D. TSAO[4];
[1]Brain & Cognitive Sci., MIT, CAMBRIDGE, MA; [2]MGH, Boston, MA; [3]Rockefeller Univ., New York, NY; [4]CalTech, Pasadena, CA

Abstract: Faces are a biologically important class of stimuli for primates. Recent work has identified six discrete face areas in the temporal lobe of the macaque that form a network which appears to be responsible for processing information related to faces (Moeller et al., 2008; Tsao et al., 2008; Freiwald and Tsao, 2010). The vast majority of neurons in these face areas have much higher firing rates to images of faces compared to other object categories (Tsao et al., 2006; Freiwald and Tsao, 2010; Issa and DiCarlo, 2012), however it is still unclear what types of information, and consequently which visual behaviors, each face area could support. In this work we use neural population decoding analyses to better characterize what information is being represented in three of these face areas (ML/MF, AL, and AM). Our decoding results show that there is more information about faces compared to non-face objects in all three regions, and that AM in particular shows a much stronger representation of faces compared to non-face stimuli. Additionally, we find that information about face identity that is invariant to the pose of the head is largely absent from ML/MF, is stronger in AL and is strongest in AM. These findings show that the face patch system builds up visual features in the more anterior patches that are useful for identifying individuals while losing information that is useful for discriminating between different non-face objects.

Disclosures: E.M. Meyers: None. **W. Freiwald:** None. **D. Tsao:** None. **M. Borzello:** None.

Nanosymposium

582. Visual Processing: Faces

Location: 143A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 582.05

Topic: D.04. Vision

Support: NSF Award 1157121

Title: Structural equation modeling reveals relationships between the left and right fusiform gyri for processing faces

Authors: *M. MENG[1], Z. LI[2];
[1]Psychological and Brain Sci., Dartmouth Col., Hanover, NH; [2]Dept. of Community and Family Med., The Geisel Sch. of Med. at Dartmouth Col., Hanover, NH

Abstract: Functional relationships between the left and right cerebral hemispheres are a fundamental but often less studied component of how the brain is organized. In the domain of face perception, the hierarchical relationship in the ventral visual pathway, e.g., from occipital face area (OFA) to fusiform face area (FFA) has been frequently studied, however, little is known about causal relationships between the left and right face areas. Previous studies have clearly identified the hemispherical asymmetry of face processing. For example, we reported previously that activity in the left FFA correlates with image-level face-semblance of stimuli whereas activity in the right FFA correlates with categorical perceptual decision of whether a visual input is a face (Meng, Cherian, Singal, & Sinha, 2012). By using a structural equation modeling analysis, the present study focuses on whether the neural responses in the left FFA and right FFA occur in parallel or whether they are serially dependent. Models were constructed based on univariate averaged fMRI activity vs. multi-voxel activation patterns in the left and right FFAs corresponding to a collection of 300 stimulus images (including 60 random nonface images, 180 false alarms from a computer face detection system, and 60 genuine face images). We then compared weights of the graphic paths that use the averaged activity (or activation pattern) in the left and right FFAs as mediators for transforming input information as feature-based face-semblance to subjective perceptual decision of face/non-face categorization. The feature-based face-semblance metric was computed for each image by detecting the presence of 12 contrast polarity relationships between face areas specified by the Sinha algorithm (Sinha, 2002), e.g. forehead is brighter than left eye and summing the number of relationships (face features) fulfilled by that image (Ohayon, Freiwald, & Tsao, 2012). We found that activation patterns in the left FFA directly contribute to perceptual decisions of face/non-face categorization. By contrast, the univariate averaged activity in the left FFA does not, but relies on activity in the right FFA as a mediator. For comparisons, both the univariate activity and activation patterns in the left FFA are directly driven by variances of face-semblance in the stimulus set. However, the right FFA relies on the left FFA as a mediator more than directly driven by image-level face-semblance, suggesting later stages of face processing in the right FFA than the left. Taken together, these results indicate a sequentially dependent relationship between the left and right FFAs for processing faces.

Disclosures: **M. Meng:** None. **Z. Li:** None.

Nanosymposium

582. Visual Processing: Faces

Location: 143A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 582.06

Topic: D.04. Vision

Support: NSF grant 0855112

NSFC grant 31230031

Title: fMRI decoding reveals impaired face configural processing in the right fusiform face area of individuals with developmental prosopagnosia

Authors: *J. ZHANG[1], J. LIU[2], Y. XU[1];
[1]Dept. of Psychology, Harvard Univ., Cambridge, MA; [2]State Key Lab. of Cognitive Neurosci. and Learning, Beijing Normal Univ., Beijing, China

Abstract: About 2% of the general population suffers from developmental prosopagnosia (DP, also known as congenital prosopagnosia), a lifelong face recognition deficit that cannot be attributed to acquired brain damage despite intact general cognitive abilities and sensory functions. In spite of the behavioral deficit in face recognition, the corresponding neural impairment in DP is not fully understood. The right fusiform face area (FFA), among the object processing regions in occipitotemporal cortex, has been showed to play a critical role in face processing. Paradoxically, DP individuals exhibit normal fMRI response amplitude profile in the right FFA, showing much higher activation for faces than objects from other categories. As previous findings have associated the right FFA with face configural processing, here using fMRI and multi-voxel pattern analysis (MVPA), we examined whether or not face configural processing is intact in the right FFA of seven individuals with DP from Beijing, China. We found that these individuals exhibited abnormal neural decoding to face configuration changes in the right FFA. Specifically, in individuals without behavioral face processing deficit, successful decoding in the right FFA was obtained between intact faces and faces whose parts were rearranged in a scrambled configuration (scrambled faces), showing the encoding of face configural information in this brain region. No such decoding, however, was obtained in the right FFA of DP individuals. This face configural decoding deficit was limited to the right FFA, as normal neural decoding was found in these DP individuals in the nearby right lateral occipital object-processing region. DP individuals also exhibited normal face response amplitude profile and largely intact neural decoding to the different face parts in the right FFA. These findings were replicated with a subset of the DP individuals in a follow-up study when Caucasian, instead

of Asian, faces were used as stimuli. Thus, in DP individuals, despite right FFA's preference to face stimuli and its ability to form unique representations for the different face parts, it failed to extract face configural information to form distinct representations for the intact and the scrambled faces. To our knowledge, this is the first direct neural evidence showing that, in DP individuals, face configural processing is impaired in the right FFA. We propose that such impairment may play a central role in behavioral face processing deficits observed in DP individuals.

Disclosures: J. Zhang: None. **J. Liu:** None. **Y. Xu:** None.

Nanosymposium

582. Visual Processing: Faces

Location: 143A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 582.07

Topic: D.04. Vision

Support: NIH 1 RO1 EY 02231801A1

facessvep 284025

Belgian National Fund for Scientific Research

Title: The resiliency of cortical networks: Stable functional organization of the face processing network after surgical resection of the right inferior occipital gyrus

Authors: *K. S. WEINER[1], L. MAILLARD[2], J. JONAS[2], H. BRISSART[2], G. HOSSU[3], C. JACQUES[4], D. LOFTUS[1], J. GOMEZ[1], K. GRILL-SPECTOR[1], B. ROSSION[4]; [1]Stanford Univ., STANFORD, CA; [2]Neurol., [3]Ctr. d'Investigation Clinique-Innovation Technologique, Univ. Hosp. of Nancy, Nancy, France; [4]Psychological Res. Inst. and Inst. of Neurosci., , Univ. of Louvain, Louvain, Belgium

Abstract: The prevailing neurofunctional model of human face perception considers the inferior occipital gyrus ("IOG-faces/OFA") the key input to a hierarchical network of face-selective regions, with a right hemisphere dominance. In a unique patient (SP), we used functional magnetic resonance imaging (fMRI), intracerebral recordings with depth electrodes (sEEG), and diffusion weighted imaging (DWI) to assess the structure and function of the face network before and after surgical resection of the face-selective IOG in the right hemisphere. Critically, we acquired fMRI measurements twice pre-resection and twice post-resection in order to compare

the effect of resection to the baseline session-to-session variability before surgery. Pre-resection, a block design fMRI experiment using images of faces, body parts, places, and objects, showed that SP had a typical network of face-selectivity with face-selective regions distributed across the IOG, fusiform gyrus (FG), and superior temporal sulcus (STS). SEEG recordings revealed the highest face-selective response in the high gamma frequency range in an electrode located in the posterior FG (pFus-faces/FFA-1) with a normal latency of face selectivity. fMRI conducted 1 month and 8 months post-resection revealed that the resection included all of IOG-faces/OFA and the posterior aspect of pFus-faces/FFA-1. However, despite the resection, FG and STS face-selective regions were remarkably stable both 1 and 8 months post-resection. DWI measurements revealed two white matter tracts - one connecting early visual areas to the posterior FG and another connecting early visual areas to the STS - which may serve as independent routes bypassing IOG-faces/OFA and may enable stable function of downstream face-selective regions on the FG and STS. Altogether, these measurements pose important constraints on the proposed hierarchical neurofunctional model of face-selective responses in the human brain, as well as provide powerful insight into the resiliency of functional networks within higher sensory cortices.

Disclosures: **K.S. Weiner:** None. **L. Maillard:** None. **J. Jonas:** None. **H. Brissart:** None. **G. Hossu:** None. **C. Jacques:** None. **D. Loftus:** None. **J. Gomez:** None. **K. Grill-Spector:** None. **B. Rossion:** None.

Nanosymposium

582. Visual Processing: Faces

Location: 143A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 582.08

Topic: D.04. Vision

Support: NIH NCCTS 5TL1TR00036907

NIH CCTS KL2 RR0224149

NIH CCTS UL1RR024148

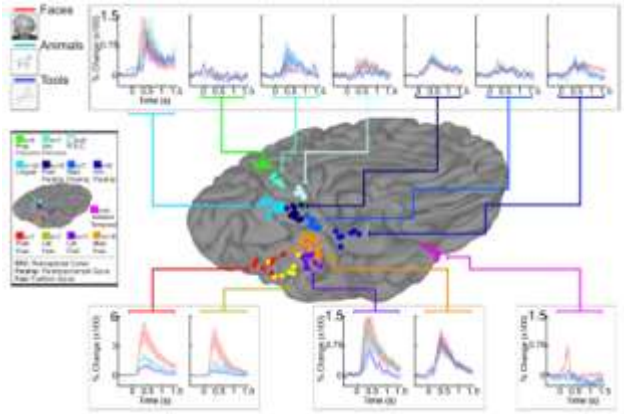
NIDA R01DA026452

NINDS R01NS076856

Title: Distributed neural systems for category-specific visual processing

Authors: *N. TANDON, C. KADIPASAOGLU, V. BABOYAN;
Neurolog. Surgery, Univ. Texas Med. Sch., HOUSTON, TX

Abstract: The debate between modular and distributed neural systems for category-specific visual processes has remained unresolved due to limitations in non-invasive neuroimaging methods. The spatio-temporal characteristics of electro-corticography (ECoG) data provide a unique opportunity to yield novel insights into cognitive operations such as category-specificity of the ventral visual cortex. However, the broader application of ECoG has been confounded by difficulties in accurately representing individual data, as well as performing statistically valid population-level analyses. To overcome these limitations, we developed methods for accurately registering ECoG data to individual cortical topology. We integrated this technique with surface-based co-registration and a mixed-effects multilevel analysis (MEMA) to perform grouped analysis, controlling for cortical variability, sparse coverage across patients, and intra- and inter-subject variability. We applied this Surface-Based MEMA (SB-MEMA) technique to visual naming tasks (tools, animals, and famous faces), performed in patients implanted with subdural electrodes for the treatment of pharmacologically resistant epilepsy (30 subjects, 17 LH, 8 RH, 5 Bilateral). Percent power change was computed in the mid-gamma range (60-120 Hz) following stimulus onset (50 to 700 ms), compared to baseline (-850 to -200 ms). To investigate temporal activation profiles, the loci of peak activity from the grouped results were used to identify corresponding electrodes across individuals and compute a grouped time series. SB-MEMA yielded significant, and overlapping power changes in the ventral and lateral occipito-temporal cortices, consistent with results from individual data. Time series analysis revealed prominent early activity (<150 ms) for all tasks, with substantial temporal overlap across cortical regions. Between categories, significant differences were revealed in the peak, but not the onset of activation. These results support the existence of a distributed neural system for visual processing of categories.



Disclosures: **N. Tandon:** None. **C. Kadipasaoglu:** None. **V. Baboyan:** None.

Nanosymposium

582. Visual Processing: Faces

Location: 143A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 582.09

Topic: D.04. Vision

Support: NSF Grant BCS-0617688

NIH Grant 1R21EY017741

NSF Grant BCS 0920865

Title: Face selectivity and representation of central vision develop in tandem along the fusiform gyrus among children

Authors: *G. GOLARAI, A. LIBERMAN, K. GRILL-SPECTOR;
Psych, Stanford Univ., STANFORD, CA

Abstract: In adults face-selective regions across the ventral temporal cortex (VTC) overlap with representations of central visual field, and place-selective regions with peripheral, perhaps due to the habitual patterns of veiwing faces with central and places with peripheral vision. Thus one hypothesis suggests that cortical representations of center and periphery develop as a result of visual experience and in spatial register with face and place selectivity. We previously reported the slow and differential development of face and place selective regions, where by place selective regions become adult like by teens, but face-selective regions developed more slowely well into the teens. However, the developmental time course of VTC's center-periphery organization or its spatial relation to the developing category selective regions are unknown. Thus, we examined the development of center-periphery organization in relation to face and place selective regions in children (ages 8 - 10 , n = 7), teens (ages 12 -16, n =13) and adults (ages 18 -40, n= 9). During fMRI, subjects viewed achromatic images of faces and places, presented at 2 eccentricities: centrally (spanning 3°), or peripherally (within a 12-24° ring), in 8 blocks/condition. Peripheral images were enlarged to account for lower peripheral acuity. Subjects fixated on a central point and pressed a button when it changes color, while an eye tracker monitored their fixation. After validating fixation performance, in each subject we mapped eccentricity bias by contrasting responses for central vs. peripheral stimuli (and vice-versa) and examined eccentricity maps in relation to face and place selectivity. Our preliminary data indicate that adults and children were similar in maintaining fixation, and the level of peripheral bias in place-selective regions. In contrast to adult's foveal bias in face-selective regions of the mid fusiform gyrus, children showed no foveal bias in this region or its vicinity.

However, this foveal bias was adult like by adolescence, even as the spatial extent of face-selective regions continued to develop. Thus, our findings todate suggest that development of face selectivity and foveal bias temporally and spatially overlap during childhood, consistent with the center-periphery hypothesis.

Disclosures: G. Golarai: None. **A. Liberman:** None. **K. Grill-Spector:** None.

Nanosymposium

582. Visual Processing: Faces

Location: 143A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 582.10

Topic: D.04. Vision

Support: Attias Family Foundation NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation (A.S.G.)

NIH Grant UL1TR000005

Title: Dynamic encoding of face information in human fusiform

Authors: *A. S. GHUMAN[1], N. M. BRUNET[1], Y. LI[1], R. O. KONECKY[1], J. A. PYLES[2], V. DESTEFINO[1], W. WANG[3], R. M. RICHARDSON[1];
[1]Neurolog. Surgery/Neurobiology, UPMC Dept. of Neurolog. Surgery, Pittsburgh, PA; [2]Carnegie Mellon Univ., Pittsburgh, PA; [3]UPMC Dept. of Physical Med. and Rehabil., Pittsburgh, PA

Abstract: Humans' ability to rapidly and accurately detect, identify, and classify faces under variable conditions derives from a network of brain regions highly tuned to face information. One of the most face selective regions in the brain is located in the fusiform gyrus (the fusiform face area, FFA) and damage to FFA results in profound impairments in face recognition. Cognitive and neural models suggest that face perception occurs via a set of dynamic information processing stages. Determining the neural underpinnings of these processing stages has proven elusive, and, specifically, the temporal dynamics of information encoding during these processes remain unclear. Here we show that FFA contributes to face detection, face individuation, and task-related face processing. Specifically, we use multivariate pattern classification to decode the timecourse of face information processing using electrodes placed directly upon FFA in humans. Early FFA activity (50-300 ms) reliably predicted whether

participants were viewing a face. Activity between 200- 500 ms contained expression-invariant information about which of 70 faces participants were viewing. During this time period, FFA activity also contained information regarding individual differences in critical facial features (eyes and mouth) and their configurations (eye-mouth ratio). Late, long-lasting (500+ ms gamma band activity contained information regarding task performance. These results elucidate the dynamic computational role FFA plays in analyzing face information through multiple stages of face processing and indicate what information FFA is sensitive to in performing these visual analyses.

Disclosures: **A.S. Ghuman:** None. **N.M. Brunet:** None. **Y. Li:** None. **R.O. Konecky:** None. **J.A. Pyles:** None. **V. Destefino:** None. **W. Wang:** None. **R.M. Richardson:** None.

Nanosymposium

582. Visual Processing: Faces

Location: 143A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 582.11

Topic: D.04. Vision

Support: IRP of NINDS, NIH

IRP of NIMH, NIH

Title: Face-selective regions of the marmoset extrastriate visual cortex - revealed by fMRI & electrocorticography

Authors: ***C.-C. HUNG**[1,2], C. C. YEN[1], J. L. CIUCHTA[1], J. R. DAY-COONEY[2], D. PAPOTI[1], N. A. BOCK[3], B. E. RUSS[2], D. A. LEOPOLD[2], A. C. SILVA[1];
[1]NINDS, NIH, Bethesda, MD; [2]NIMH, NIH, Bethsda, MD; [3]McMaster Univ., Hamilton, ON, Canada

Abstract: Primates rely upon vision in their social interaction, perhaps most notably in the reading of faces to determine the identity, emotional state, and attentional focus. Multiple regions of the occipitotemporal visual cortex of humans and macaques appear specialized for the visual processing of faces. The homological correspondence of the face patches is a matter of speculation, in part because there is little information on how such circuits evolved. Using fMRI and electrocorticography (ECoG), previously we demonstrated that the common marmoset (Callithrix jacchus), a small New World monkey possesses a network of six face-selective

cortical areas mainly in and lateral to the superior temporal sulcus (STS) that closely resembles those in humans and macaques. Here, we attempt to better characterize the functional properties of each face areas to establish the homology. We trained four marmosets to direct their gaze toward presentations of static images or movie clips. Eye position was monitored and behavioral reinforcement for maintenance of gaze was achieved using liquid reward. In two of the animals, we measured fMRI responses in a 7T horizontal scanner (Bruker AVANCE AVIII). Whole brain Blood oxygenation level-dependent (BOLD) responses were acquired at a spatial resolution of 0.5x0.5x0.5 mm. In the other two animals, we implanted pairs of 32-channel ECoG arrays with 1mm inter-site distance in the occipitotemporal cortex; the arrays were able to cover continuous stretch of occipitotemporal visual pathway spanning from V2 to TE because of marmoset's lissencephaic cortex. The electrophysiological signals complemented the fMRI measures by providing higher temporal resolution and access to more ventral cortical areas. The high-gamma band (50-150Hz) ECoG responses showed a clear posterior-anterior gradient of increase sensitivity to structured versus scrambled images, which could easily be visualized along the ventral pathway without interruption by the sulci. Moreover, the ECoG data revealed a shorter response latency for a dorsal face patch in STS compared to that of the face areas in more lateral and ventral TE. The latency differences support the emerging notion of dorsal and ventral pathways for face processing, broadly consistent with similar findings in macaques and humans. In a complementary set of experiments, we measured fMRI responses of marmosets and macaques viewing the same set of natural movies. Applying inter-species alignment of the time courses to this data provides an additional perspective on areal homology of the face patches.

Disclosures: C. Hung: None. **C.C. Yen:** None. **J.L. Ciuchta:** None. **J.R. Day-Cooney:** None. **D. Papoti:** None. **N.A. Bock:** None. **B.E. Russ:** None. **D.A. Leopold:** None. **A.C. Silva:** None.

Nanosymposium

582. Visual Processing: Faces

Location: 143A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 582.12

Topic: D.04. Vision

Support: NIH (R01EY019702)

National Science Foundation (BCS-0847798)

Institutional start-up, Conte Center Grant

Title: Representation of a parameterized realistic face space across different face patches

Authors: *L. CHANG, D. TSAO;
Div. of Biol., CALIFORNIA INSTITUTE OF TECHNOLOGY, Pasadena, CA

Abstract: The macaque temporal lobe contains six patches of face-selective cortex. Previous studies demonstrate neurons in these patches show selectivity for individual identity, and experiments with parameterized cartoon faces have revealed some of the dimensions for coding faces in the middle face patches (such as face aspect ratio, eye size etc.; Freiwald, Tsao and Livingstone, 2009), but it remains unclear how face cells in different patches work together to encode realistic faces. Do different patches encode different facial dimensions? How well can one reconstruct a face based on population activity in each face patch? To address these questions, we generated a parametric real face space defined by 25 shape and 25 appearance dimensions. To construct this face space, we started with a database of 200 frontal faces (FEI face database), and for each face, used an "active appearance model" (a model developed in the field of computer vision which considers both shape and appearance information of facial images, for details see Cootes, Edwards and Taylor, 2001) to extract significant facial features describing both the shape and appearance; we then computed the top 25 principal components for shape and for appearance. Realistic facial images were then generated by randomly assigning values to each of the 50 dimensions. Neural responses of face cells were recorded while a set of 2000 such images were presented sequentially, and the data was analyzed using spike-triggered averaging. For most face cells recorded, we found the responses were significantly and monotonically modulated by the face dimension determined by the spike-triggered average (STA) stimulus. Across the population of recorded face cells, the shapes of STAs were very diverse. We are now investigating systematic differences in facial-feature tuning across different face patches. We compared the previously characterized view-invariant sparsely firing face cells in the most anterior face patch AM with other non-sparse face cells, and found that the relationship between firing rate and linear STA response displayed stronger nonlinearity (thresholding) in sparse cells.

Disclosures: L. Chang: None. **D. Tsao:** None.

Nanosymposium

582. Visual Processing: Faces

Location: 143A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 582.13

Topic: D.04. Vision

Support: NIH NCCTS 5TL1TR000369-07

NIH CCTS KL2 RR0224149

NIH CCTS UL1RR024148

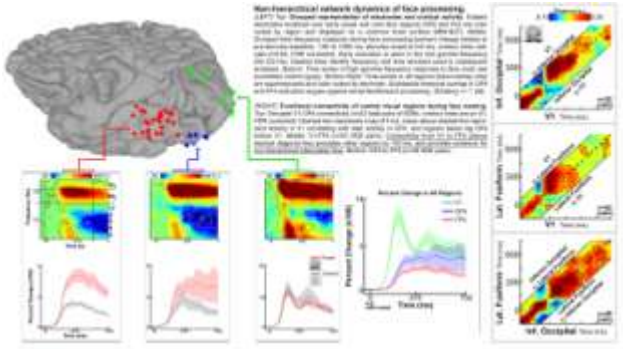
NIDA R01DA026452

NINDS R01NS076856

Title: Non-hierarchical network dynamics of face perception

Authors: *C. M. KADIPASAOGLU, C. CONNER, V. BABOYAN, N. TANDON; Neurosurg., Univ. of Texas Med. Sch. At Houston, Houston, TX

Abstract: Face perception has long been understood to occur through a feed-forward, hierarchical network in the ventral visual cortex: low-level feature detection (e.g. eyes) is performed by the more inferior occipital gyrus (occipital face area, OFA), which then outputs information to the posterolateral fusiform gyrus (fusiform face area, FFA) for structural decoding. Recent studies, however, have demonstrated that patients suffering from uni- or bilateral OFA lesions are still able to successfully categorize faces and induce normal FFA activation. This argues for non-hierarchical processing mechanisms by which the FFA operates independent of OFA input, potentially through direct connections with early visual cortex. Resolution between these competing hypotheses is difficult due to limitations in spatio-temporal resolution of non-invasive neuroimaging methods. We leveraged millisecond resolution intracranial EEG (iEEG) data, recorded during famous face naming (11 subjects; 4 RH, 7 LH), to investigate inter-regional dynamics in the face network. Functional connectivity was assessed using amplitude envelope correlations (AEC) of high frequency activity fluctuations (HFA; 60-120 Hz; -50 to 750 ms post stimulus) between pairs of individual subject electrodes located over early visual (V1/V2) and core face regions (OFA and FFA). Directionality of connectivity was estimated by lagging these time series. In the right hemisphere, non-hierarchical interactions were confirmed by the demonstration of unique patterns of bidirectional V1-FFA connectivity, beginning earlier than for OFA-FFA connectivity. These results provide empirical evidence in support of non-hierarchical information flow during face perception.



Disclosures: **C.M. Kadipasaoglu:** None. **C. Conner:** None. **V. Baboyan:** None. **N. Tandon:** None.

Nanosymposium

583. Brainstem: Motor and Sensory Systems

Location: 144A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 583.01

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

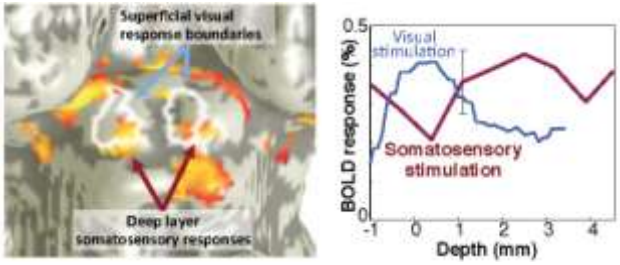
Support: NSF BCS 1063774

Title: Measurements of somatotopic organization in the deep layers of human superior colliculus

Authors: *D. RESS[1], S. KATYAL[2], C. GREENE[3];
[1]Neurosci., Baylor Col. of Med., Houston, TX; [2]Psychology, Univ. of Minnesota, Twin Cities, MN; [3]Electrical Engin., Univ. of California, Santa Barbara, CA

Abstract: The deep layers of superior colliculus (SC) respond to multiple sensory modalities, and are believed to be involved in multisensory integration. As a first step in the examination of SC multisensory function, we wish to map the representation of various somatosensory stimulation locations in the SC using high-resolution fMRI. **Methods**: In preliminary experiments, subjects ($N = 3$) performed a visually cued finger-tapping task. Based on a sequence of flashing dots on a visual display, subjects attempted to tap corresponding buttons on an input device in a simple sequence at a constant rate of 2 Hz. The subject's actual tapping rate was continuously updated on the display. Accurate performance of the task is challenging, and requires continuous attention. Tapping tasks were either bilateral (tapping vs. rest), or left-right

alternations. High-resolution (1.2 mm) interleaved spiral acquisition fMRI (3-sec sampling) was obtained using a 3T scanner throughout 4-min runs of each condition. SC was segmented to obtain a surface model, and a depth map was calculated to examine depth variations of the response. Activity was averaged through a 2—4-mm depth range to show deep-layer activity topography on the surface model, and depth profiles were obtained to examine laminar variations. **Results**: We observe significant deep-layer activity in caudo-medial portions of both colliculi (Figure). Topography was similar for both task conditions, and responses were well lateralized. Relative to the representation of retinotopy in the superficial layers of SC, the location of the somatosensory-evoked activity was consistent with a large eccentricity in the lower visual field, similar to the position of the hands in retinotopic coordinates during task performance in the scanner. **Conclusion**: Mapping of somatotopy in SC is feasible using a 3T scanner. Somatotopy shows rough agreement with retinotopy during a visually cued finger-tapping task.



Disclosures: **D. Ress:** None. **S. Katyal:** None. **C. Greene:** None.

Nanosymposium

583. Brainstem: Motor and Sensory Systems

Location: 144A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 583.02

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: MA was supported by a University of Queensland summer and winter research placement scholarship

Title: Neuromuscular correlates of midbrain periaqueductal gray induced vocalization in decerebrate cats: Principles of descending laryngeal and respiratory motor control

Authors: ***H. SUBRAMANIAN**, M. ARUN;
The Univ. of Queensland, Herston, Australia

Abstract: In humans, presently, only qualitative examination of laryngeal EMG is used as a tool to establish the extent of speech pathology. However, quantification of laryngeal and respiratory muscle EMG would aide in standardizing methodology across speech clinics in diagnostic and prognostic accuracy of speech and vocalization disorders. This requires investigation of various muscular activation properties involved in vocalization and their neuromotor control. While many studies in human otolaryngology have investigated (and continue to investigate) the larynx, animal studies that can serve as a good model are limited. Vocalizations in cats are regarded as expressions of emotional or motivational state and serve a model for the anatomy, physiology and pharmacology of neural processing and human diseases associated with speech disorders. In this study, I topographically mapped the midbrain periaqueductal gray (PAG) for producing three different types of known vocalization, the mew, the howl and the hiss. I investigated the muscular correlates of PAG induced mew, howl and hiss via recording the recruitment patterns of the laryngeal (posterior cricoarytenoid, cricothyroid and thyroarytenoid), respiratory (internal and external abdominal obliques, internal intercostals, the crural and costal diaphragm), the genioglossus (tongue) and the digastric (mouth opening) muscles. Mews and howls entailed activation of the cricothyroid and thyroarytenoid muscles together with inhibition of the posterior cricoarytenoid, whereas the hiss is characterized by activation of thyroarytenoid and posterior cricoarytenoid muscle while the cricothyroid did not show any significant activity. Mews and howls were also associated with strong activation of the external and internal oblique and internal intercostal expiratory muscles, while during hisses the oro-facial, digastric, and genio-glossus muscles were excited. This data provides a basis for investigation of descending laryngeal and respiratory motor control pathways and neuromotor principles underlying synergy of laryngeal and respiratory modulation during specific types of vocalization. This also serves to study neuromotor components of human speech and language representation such as vowels and consonants in health and disease.

Disclosures: H. Subramanian: None. **M. Arun:** None.

Nanosymposium

583. Brainstem: Motor and Sensory Systems

Location: 144A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 583.03

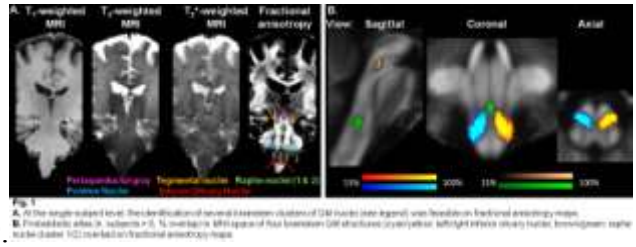
Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH NIBIB 5 P41 RR014075-13

Title: *In vivo* delineation of human brainstem grey matter with Diffusion Tensor Imaging at 7 Tesla

Authors: *M. BIANCIARDI[1,2], N. TOSCHI[2,3], C. EICHNER[2], B. EDLOW[4], J. R. POLIMENI[2], K. SETSOMPOP[2], D. BOAS[2], L. L. WALD[2];
[1]MGH & HMS, Cambridge, MA; [2]Dept. of Radiology, Athinoula A. Martinos Ctr. for Biomed. Imaging, Massachusetts Gen. Hosp. and Harvard Med. Sch., Boston, MA; [3]Med. Physics Section, Dept. of Biomedicine and Prevention, Fac. of Medicine, Univ. of Rome "Tor Vergata", Rome, Italy; [4]Dept. of Neurology, Athinoula A. Martinos Ctr. for Biomed. Imaging, Massachusetts Gen. Hosp., Boston, MA

Abstract: Introduction: The human brainstem plays an important role in several vital functions, including arousal, nociception, autonomic homeostasis, and sensory functions. Our current knowledge of grey matter (GM) structure within the brainstem mostly derives from *ex vivo* studies [1-2]. Aim of this work was to develop a novel *in vivo* MRI tool to identify brainstem GM structures. **Methods:** Eight subjects (6m/2f, age 28 ± 2) participated in this IRB approved study. We employed *in vivo* high spatial-resolution (1.1 mm isotropic) diffusion tensor imaging (DTI) at 7 Tesla (60 directions, b ~ 1000 s/mm^2, 7 interspersed b0 images, TE/TR = 60.8/5600 ms, 4 repetitions), and scrutinized the contrast in DTI-maps, including fractional-anisotropy (FA), computed after eddy-current correction and tensor estimation. We also acquired distortion- and resolution-matched T_2-, T_2*-weighted images and a 1 mm^3 T_1-weighted MPRAGE. On a single-subject basis, FA maps were automatically segmented by k-means clustering. Labels of 4 brainstem GM structures (raphe nuclei cluster 1 and 2, left and right inferior olivary nuclei clusters) were mapped and normalized to MNI space through high dimensional non-linear transformations, and a probabilistic atlas for these structures was created. **Results:** In single subject FA maps, major clusters of brainstem GM structures were visible with higher contrast compared to T_1-, T_2-, and T_2*-weighted MRI (Fig.1A). The raphe nuclei cluster 1 and the inferior olivary nuclei displayed higher overlap across subjects than the raphe nuclei cluster 2. **Conclusions:** High spatial resolution DTI at 7 Tesla enabled the delineation of brainstem GM structures on a single subject basis. Our results also demonstrate the feasibility of developing a probabilistic atlas of brainstem GM structures, which could be used as a tool to perform functional and structural connectivity studies of human brainstem GM in health and disease.
References: [1] Paxinos and Huang, Atlas of the human brainstem, Academic Press, 1995. [2] Naidich, Duvernoy's atlas of the human brainstem and cerebellum, Springer, 2009.



Disclosures: **M. Bianciardi:** None. **N. Toschi:** None. **C. Eichner:** None. **B. Edlow:** None. **J.R. Polimeni:** None. **K. Setsompop:** None. **D. Boas:** None. **L.L. Wald:** None.

Nanosymposium

583. Brainstem: Motor and Sensory Systems

Location: 144A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 583.04

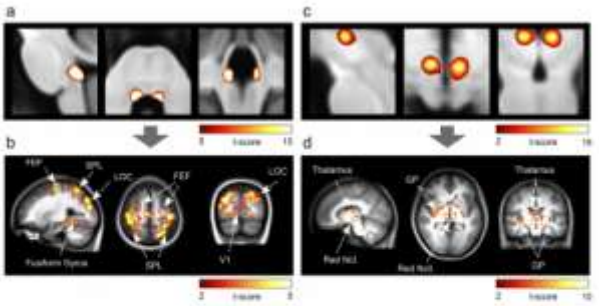
Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Title: Identification of brainstem nuclei in resting-state fMRI data based on their cortical connectivity profiles

Authors: ***F. BEISSNER**[1], A. SCHUMANN[2], F. BRUENNER[2], K.-J. BÄR[2];
[1]Neuroradiology, Somatosensory and Autonomic Therapy Res., Hannover, Germany; [2]Univ. Hosp. Jena, Jena, Germany

Abstract: The brainstem is the most important part of the brain, when it comes to sustaining our life. Despite its tremendous importance, it has been largely neglected by human neuroscience. One of the major reason for this neglect lies in the poor performance of standard neuroscientific measurement methods, like functional magnetic resonance imaging (fMRI), in this part of the brain, which is mainly due to the elevated level of physiological noise. Recently, we introduced a new method to detect activity of brainstem nuclei from resting-state fMRI data using a masked independent component analysis (mICA), where a probabilistic ICA is restricted to the volume inside an antomical brainstem mask, thus, suppressing physiological noise in the spatial domain (Beissner et a., 2014)□. While brainstem resting-state components detected by mICA have been shown to be reproducible and specific, their anatomical identification and functional interpretation remains a challenging problem. Using a sample of 100 healthy volunteers measured with iPAT-accelerated echo-planar imaging on a 3-Tesla MRI scanner, we show how the mICA approach can be expanded to study functional connectivity between brainstem nuclei and other structures of the brain. Cortical and subcortical connectivity profiles are derived by dual regression of the nuclei's time-courses onto the whole-brain functional data. We show how these profiles can be used, both, to confirm anatomical identification of known brainstem nuclei as well as to gain a functional interpretation of previously unknown brainstem resting-state networks derived from low-dimensional mICA analyses. Among others, our approach was able to detect nuclei of the somatosensory, autonomic, vestibular, auditory and neuromodulatory systems. The presented method introduces a new way to investigate functional connectivity

between brainstem nuclei and their associated cortical regions. Reference: Beissner, F., Schumann, A., Brunn, F., Eisenträger, D., & Bär, K. (2014). Advances in functional magnetic resonance imaging of the human brainstem. Neuroimage, 86, 91-98.



Brainstem-Cortex functional connectivity of two well-known nuclei: The abducens nucleus (a) and the red nucleus (c). Their time-courses were used in a dual regression onto the whole-brain data to identify functionally connected regions in cortical areas (b+d). The connectivity profile of the abducens nucleus (b) contains mainly regions that are associated with vision, attention and eye movements. For the red nucleus, connectivity was observed mainly with the globus pallidus of the lenticular nucleus, with the thalamus as well with two cortical regions in the orbito-frontal cortex and precuneus. FEF: frontal eye fields, GP: globus pallidus, LOC: lateral occipital cortex, MTG: medial temporal gyrus, OFC: orbitofrontal gyrus, SPL: superior parietal lobule, V1: striate cortex.

Disclosures: **F. Beissner:** None. **A. Schumann:** None. **F. Bruenner:** None. **K. Bär:** None.

Nanosymposium

583. Brainstem: Motor and Sensory Systems

Location: 144A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 583.05

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: JSPS KAKENHI Grant Number 24300108

JSPS KAKENHI Grant Number 24500365

JSPS KAKENHI Grant Number 25540130

JSPS KAKENHI Grant Number 23650218

JSPS KAKENHI Grant Number 26460311

JSPS KAKENHI Grant Number 26670676

Health Labour Sciences Research Grant

Title: Connectomics of the inspiratory center: Anatomical tract and propagation dynamics from the preBötzinger complex

Authors: *Y. OKADA[1], Y. OKU[2], S. YOKOTA[3], Y. OYAMADA[4], Y. YASUI[3], N. KOSHIYA[5];
[1]Murayama Med. Ctr., Tokyo, Japan; [2]Dept. of Physiol., Hyogo Col. of Med., Nishinomiya, Japan; [3]Dept. of Anat. & Molphol. Neurosci., Shimane Univ. Sch. of Med., Izumo, Japan; [4]Dept. of Respirol., Tokyo Med. Ctr., Tokyo, Japan; [5]NIH - NINDS, Bethesda, MD

Abstract: Maintenance of respiratory rhythm is essential to life. It has been accepted that the preBötzinger complex (preBötC) of the ventrolateral medulla is the kernel for inspiratory rhythm generation. However, it is not fully understood how inspiratory neural activity is generated in the preBötC and propagates to other medullary regions. We analyzed the detailed anatomical connectivity to and from the preBötC and functional aspects of the inspiratory information propagation from the preBötC on the transverse plane of the medulla oblongata. Neuroanatomical tract-tracing with immunohistochemistry in young adult rats demonstrated that neurokinin-1 receptor- and somatostatin-immunoreactive neurons in the preBötC, which could be involved in respiratory rhythmogenesis, are embedded in the plexus of axons originating in the contralateral preBötC. By voltage-imaging in rhythmically active slices of neonatal rats, we analyzed origination and propagation of inspiratory neural activity as depolarizing wave dynamics on the entire transverse plane as well as within the preBötC. Novel combination of pharmacological blockade of glutamatergic transmission and mathematical subtraction of the video images under blockade from the control images enabled to extract glutamatergic signal propagations. By ultra-high-speed voltage-imaging we have demonstrated inter-preBötC conduction of action potentials for the first time. Intra-preBötC imaging with high spatiotemporal resolution during single spontaneous inspiratory cycle unveiled deterministic nonlinearities, i.e., chaos, in the population recruitment. We comprehensively elucidated the anatomical pathways to and from the preBötC and dynamics of inspiratory neural information propagation: (1) from the preBötC in one side to the contralateral preBötC, which would synchronize the bilateral rhythmogenic kernels, (2) from the preBötC directly to the bilateral hypoglossal premotor and motor areas as well as to the nuclei tractus solitarius, and (3) from the hypoglossal premotor areas toward the hypoglossal motor nuclei. The coincidence of identified anatomical and functional connectivity between the preBötC and other regions in adult and neonatal rats, respectively, indicates that this fundamental connectivity is already well developed at the time of birth.

Disclosures: **Y. Okada:** None. **Y. Oku:** None. **S. Yokota:** None. **Y. Oyamada:** None. **Y. Yasui:** None. **N. Koshiya:** None.

Nanosymposium

583. Brainstem: Motor and Sensory Systems

Location: 144A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 583.06

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NRF-2012R1A3A2048834

NRF-2012R1A1A1042191

2012-0009328

22300127

Title: Enhancement of INaP-mediated resonance by mGluR-I activation induces burst firing in mesencephalic trigeminal sensory neurons

Authors: Y. KANG[1], *G. CHUNG[2], M. SAITO[1], M. TAKADA[4], Y. BAE[5], J.-S. KIM[2], S. OH[3]; [1]Grad. Sch. of Dent., Osaka Univ., Osaka, Japan; [2]Neurobio. & Oral Physiol., [3]Brain & Cognitive Sci., Seoul Natl. Univ., Seoul, Korea, Republic of; [4]Primate Res. Inst., Kyoto Univ., Inuyama, Japan; [5]Oral Anat., Kyungpook Natl. Univ., Daegu, Korea, Republic of

Abstract: The primary sensory neurons supplying the muscle spindle of jaw-closing muscles are unique in that they have their somata in the mesencephalic trigeminal nucleus (MTN) in the brainstem, thereby receiving various synaptic inputs. MTN neurons display burst firings upon activation of glutamatergic synaptic inputs while they faithfully relay respective impulses arising from peripheral sensory organs. The persistent sodium current (INaP) is reported to be responsible for both the generation of bursts and the relay of impulses. We addressed how INaP is controlled either to trigger bursts or to relay respective impulses as single spikes. Protein kinase C (PKC) activation in MTN neurons enhanced INaP only at low voltages. Both the bursting and single spiking were either facilitated or suppressed by PKC activation depending on the baseline membrane potential or on the presence or absence of 4-aminopyridine. By injection of a ramp current pulse, a burst of spikes was triggered from a depolarized membrane potential whereas its instantaneous spike frequency (ISF) remained almost constant despite increases in the current amplitude during the ramp pulse beyond the threshold. Activation of PKC or mGluR1/5 by dihydroxyphenylglycine (DHPG) lowered the threshold for bursting, leaving the ISF unchanged. DHPG increased both the frequency and impedance of membrane resonance in MTN neurons. Puff application of AMPA and glutamate induced burst firing. Glutamate-induced bursts were more prolonged than AMPA-induced bursts. Immunohistochemical examination

revealed that glutamatergic synapses are made onto the stem axons, and that mGluR1/5 and Nav1.6 are co-localized in the stem axon. Taken together, glutamatergic synaptic inputs onto the stem axon may be able to switch the relaying to the bursting mode.

Disclosures: **Y. Kang:** None. **G. Chung:** None. **M. Saito:** None. **M. Takada:** None. **Y. Bae:** None. **J. Kim:** None. **S. Oh:** None.

Nanosymposium

583. Brainstem: Motor and Sensory Systems

Location: 144A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 583.07

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: CIHR MOP-4918

Faculty of Dentistry, University of Toronto

Title: Loss of teeth in genetically-mapped recombinant inbred mouse strains as a model to study the genetic control of orofacial sensorimotor functions and the associated functional and sMRI-defined plasticity of the orofacial sensorimotor cortex post-injury

Authors: *L. AVIVI-ARBER[1], M. FRIEDEL[4], J. LERCH[4], Y. HAYASHI[5], G. LANDZBERG[2], M. MOAYEDI[6], K. D. DAVIS[3], Z. SELTZER[7], B. J. SESSLE[2]; [1]Fac. of Dentistry, Dept. of Prosthodontics, [2]Fac. of Dent., [3], Univ. Hlth. Network, Toronto Western Res. Inst., Univ. of Toronto, Toronto, ON, Canada; [4]Mouse Imaging Ctr., Hosp. for Sick Children, Toronto, ON, Canada; [5]Dept. of Removable Prosthodontics, Nihon Univ. Sch. of Dent. at Matsudo, Sakaecho-Nishi Matsudo Chiba, Japan; [6]Dept. of Neuroscience, Physiol. and Pharmacol., Univ. Col. London, London, United Kingdom; [7]Fac. of Dent., Univ. of Toronto Ctr. for the Study of Pain, Toronto, ON, Canada

Abstract: Rationale & Aim: Tooth loss causes orofacial sensorimotor alterations that are highly variable across individuals, suggesting that these traits are under genetic control. Our aim was to develop a model to identify genes controlling plasticity of the mammalian orofacial sensorimotor cortex (oSMCx) after orofacial injury. The facial whisker pad is innervated by afferents not injured by extraction of maxillary molar teeth and is extensively represented in oSMCx. Thus, 'extra-territorial' post-extraction sensory changes contrasting across strains of mice could model the genetic control of oSMCx plasticity. We also tested if this plasticity could be detected with

high-resolution structural magnetic resonance imaging (sMRI). The AXB-BXA panel of 23 mice strains, derived by recombinations from inbred A/J and C57BL/6J progenitors, has been genetically mapped, and thus phenotypic strain differences can be used to map quantitative trait loci harbouring genes that control these traits. Methods: BXA14 (N=10) and BXA24 (N=21) adult female mice were chosen for this pilot study since we have previously shown contrasting tactile sensitivity post-injury in these 2 strains. Facial tactile sensitivity was determined with von Frey monofilaments at 3 days pre-, and 4, 7, 14 & 21 days following right maxillary molar teeth extraction or sham operation. Baseline-normalized post-treatment withdrawal thresholds were integrated per mouse by using 30-polynomial curve fitting. Post-mortem whole-brain 7T sMRI was carried out on mice perfused at post-extraction day 21 to examine regional cortical volumes. Results: BXA24 mice had significantly higher facial tactile sensitivity than BXA14 mice (RM-ANOVA followed by Holm-Sidak test, $p<0.001$). Compared to sham operation, teeth extraction caused tactile hyposensitivity in BXA24 mice (Mean ± SEM: -29.7±39.6g vs 98.9±30.5g, respectively; $p=0.022$) but not in BXA14 mice (186.5±96.5g vs 180.6±46.1g, respectively, $p=0.082$). Extraction also caused regional local volume decreases in the oSMCx in both mouse strains (significant at 10% FDR), consistent with our previous findings of functional plasticity reflected in decreased jaw and tongue motor representations in rat oSMCx following teeth extraction (SfN 2011). Conclusions: Teeth extraction in mice produces genetically-controlled facial sensory changes associated with oSMCx structural plasticity. These data justify completing the phenotyping of all AXB-BXA strains to map genes controlling orofacial sensorimotor functions and sMRI-defined plasticity, and also highlight the utility of high-resolution sMRI for studying injury-induced oSMCx plasticity in small mammals.

Disclosures: **L. Avivi-Arber:** None. **M. Friedel:** None. **J. Lerch:** None. **Y. Hayashi:** None. **G. Landzberg:** None. **M. Moayedi:** None. **K.D. Davis:** None. **Z. Seltzer:** None. **B.J. Sessle:** None.

Nanosymposium

583. Brainstem: Motor and Sensory Systems

Location: 144A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 583.08

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Title: Cerebral cortical projections to trigeminal premotoneurons controlling jaw-movements

Authors: *A. YOSHIDA, K. TSUTSUMI, F. SATO, H. OHARA, T. KATO;
Dept. Oral Anat/ Neurobiol, Osaka Univ. Grad. Sch. of Dent., Osaka 565-0871, Japan

Abstract: In the rat, the trigeminal premotoneurons (interneurons directly project to the trigeminal motor nucleus [Vmo] which contains jaw-closing [JC] and jaw-opening [JO] motoneurons) are widely distributed in the pons and medulla which include the intertrigeminal region (Vint), trigeminal mesencephalic nucleus (Vmes), reticular formation medial to the JO component of the Vmo (rmJO), juxtatrigeminal region (Vjuxt), trigeminal oral subnucleus (Vo), and solitary tract nucleus (Sol). Of these premotoneuron areas, the Vint and Vmes mainly contain the JC premotoneurons, while the rmJO mainly contains the JO premotoneurons. The Vjuxt, Vo and Sol contain both types of the premotoneurons. Recently we examined characteristics of projections from the cerebral cortex to these premotoneuron areas by using neuronal tract tracing methods in rats anesthetized deeply with sodium pentobarbital (55 mg/kg, i.p.). The Vint received projections mainly from the lateral part of agranular cortex (Agl) which possibly corresponds to the primary somatomotor cortex (M1), while the rmJO received projections mainly from the medial part of agranular cortex (Agm) which possibly corresponds to the secondary somatomotor cortex (M2) and is also considered to be a part of the prefrontal cortex; the prefrontal cortex is involved in the autonomic and limbic function. The Vjuxt and Vo received projections mainly from the primary somatosensory cortex (S1) and Agl. The Vmes received projections mainly from the lateral part (insular cortex) and medial part of the prefrontal cortex, while the Sol received projections mainly from the insular cortex. These findings suggest that the trigeminal premotoneurons except for those in the rmJO can be divided into two groups which respectively receive projections mainly from the somatic sensorimotor cortex and from the prefrontal cortex, indicating that each premotoneuron area possibly has a distinct functional significance in the central neuronal mechanisms underlying the jaw-movements.

Disclosures: **A. Yoshida:** None. **K. Tsutsumi:** None. **F. Sato:** None. **H. Ohara:** None. **T. Kato:** None.

Nanosymposium

583. Brainstem: Motor and Sensory Systems

Location: 144A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:15 PM

Presentation Number: 583.09

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Title: Histaminergic modulation of oral-motor activity

Authors: K. NAKAYAMA[1], C. GEMBA[1,2], S. NAKAMURA[1], A. MOCHIZUKI[1], M. INOUE[2], *T. INOUE[1];
[1]Dept Oral Physiol., [2]Dept Pediatric Dent., Showa Univ. Sch. Dent., Tokyo, Japan

Abstract: Hypothalamic neuronal histamine is a factor regulating feeding behaviors. It has been reported that many histamine receptors are expressed in the brain regions, such as mesencephalic trigeminal sensory nucleus (MesV) and trigeminal motor nucleus (MoV), which are involved in controlling oral-motor activity. Moreover, MesV receives dense histaminergic hypothalamic innervation. Thus, the effects of histamine on the MesV neurons and jaw-closing motoneurons that form a reflex arc for the jaw-jerk reflex were examined using whole-cell recording technique in brainstem slice preparations from Wistar rats aged between postnatal days 7-13. MesV neurons and jaw-closing motoneurons were retrogradely labeled by tetramethylrhodamine injected into the masseter muscle one to 3 days prior to the preparation of the slices. Bath-application of histamine (100 μM) induced membrane depolarizations in MesV neurons (mean 3.7 ± 0.4 mV, n = 27) and jaw-closing motoneurons (mean 8.1 ± 1.8 mV, n = 8) at the resting potential in the presence of tetrodotoxin. Similar depolarizations were observed in both MesV neurons and jaw-closing motoneurons in each case of bath-application of a H1 receptor agonist (100 μM 2-pyridylethlamine dihydrochloride), a H2 receptor agonist (100 μM dimaprit dihydrochloride) and a H3 receptor agonist (100 μM immethridine dihydrobromide). In contrast, bath-application of the H1 receptor agonist reduced the peak amplitude of the postsynaptic inward currents (PSCs) in the jaw-closing motoneurons evoked by electrical stimulation of the MesV and the trigeminal nerve tract to 53 ± 3.3 % of the control (n = 12) and 54 ± 7.4 % of control (n = 6), respectively, whereas the H2 and H3 receptor agonists had little effects on the PSCs in the jaw-closing motoneurons. These results suggest that activation of the histamine H1 receptor is involved in regulating the jaw-jerk reflex, although MesV neurons and jaw-closing motoneurons express all of histamine H1, H2 and H3 receptors.

Disclosures: K. Nakayama: None. **C. Gemba:** None. **S. Nakamura:** None. **A. Mochizuki:** None. **M. Inoue:** None. **T. Inoue:** None.

Nanosymposium

584. Early Exposure to Stress: Environmental Factors

Location: 147B

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 584.01

Topic: E.05. Stress and the Brain

Support: US EPA Children's Environmental Health Center award RD 83329301

Research Incubator Award from Duke Institute for Brain Sciences

National Science Foundation Graduate Research Fellowship

Title: Maternal stress exacerbates the effects of prenatal air pollution exposure on offspring anxiety, cognition, and neuroimmune function in a sex-specific manner

Authors: *J. L. BOLTON[1], N. C. HUFF[1], S. H. SMITH[1], N. MASON[2], M. FOSTER[3], R. L. AUTEN[2], S. D. BILBO[1];
[1]Psychology & Neurosci., Duke Univ., Durham, NC; [2]Dept. of Pediatrics, [3]Dept. of Med., Duke Univ. Med. Ctr., Durham, NC

Abstract: Low socioeconomic status is consistently associated with reduced physical and mental health, but the mechanisms remain unclear. Increased levels of urban air pollutants interacting with parental stress have been proposed to explain health disparities in respiratory disease, but the impact of such interactions on mental health is unknown. We aimed to determine whether combined prenatal air pollution exposure and stress during pregnancy act synergistically on offspring to induce a neuroinflammatory response and subsequent neurocognitive disorders in adulthood. Mouse dams were intermittently exposed via oropharyngeal aspiration to diesel exhaust particles (DEP; 50 μg x 6 doses) or a control solution throughout gestation, combined with standard housing or nest material restriction (NR; a novel model of maternal stress) during the last third of gestation. In the adult offspring, males and females of only the combined stressor group (DEP/NR) showed increased anxiety-like behavior, whereas a significant impairment in contextual memory following fear conditioning was observed only in the male offspring of this group. Maternal DEP exposure increased proinflammatory IL-1β levels within the brains of adult males, but not females, and maternal DEP and NR both decreased anti-inflammatory IL-10 in male, but not female, brains. Intriguingly, the brain cytokine levels were significantly correlated with the display of anxiety and cognitive deficits in these animals. In a separate group of animals collected at embryonic day 18, we were able to detect a sexually dimorphic cytokine response to DEP, in which male fetal brains downregulated, whereas female fetal brains upregulated, the anti-inflammatory cytokine IL-10 following maternal DEP exposure. Furthermore, only DEP/NR male brains exhibited increased expression of the innate immune recognition gene, toll-like receptor (TLR)4, and its downstream effector, caspase-1, at postnatal day 30, which may play a role in the altered cytokine milieu observed in the fetal and adult brains. In sum, these results demonstrate that maternal stress during late gestation increases the susceptibility of offspring—particularly males—to the deleterious effects of prenatal air pollutant exposure on mood and cognition in adulthood, which may be due to a synergism of these factors acting on innate immune recognition genes and downstream neuroinflammatory cascades within the developing brain.

Disclosures: **J.L. Bolton:** None. **N.C. Huff:** None. **S.H. Smith:** None. **N. Mason:** None. **M. Foster:** None. **R.L. Auten:** None. **S.D. Bilbo:** None.

Nanosymposium

584. Early Exposure to Stress: Environmental Factors

Location: 147B

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 584.02

Topic: E.05. Stress and the Brain

Support: NIH Grant MH091351

NIH Grant MH091351-S

NIH Grant HD06028

Title: Maternal interleukin-6 concentrations during pregnancy and newborn functional brain connectivity

Authors: ***C. BUSS**[1], A. M. GRAHAM[2], M. D. RUDOLPH[2], J. RASMUSSEN[3], S. ENTRINGER[1], P. D. WADHWA[3], D. A. FAIR[2];
[1]Charité Univ. Med. Berlin, Berlin, Germany; [2]Oregon Hlth. & Sci. Univ., Portland, OR; [3]UC Irvine, Irvine, CA

Abstract: Maternal gestational psychosocial and immune stress increases offspring risk for psychiatric disorders. Inflammatory cytokines represent a likely mediator for effects of maternal prenatal stress and inflammation on the developing fetal brain with implications for subsequent mental health. Maternal interleukin-6 (IL-6) is of particular interest due to evidence for increased concentrations in response to psychosocial stress and infection, and its capacity to both cross the placenta and stimulate placental cytokine production. However, effects of maternal IL-6 during pregnancy on the fetal brain have not been reported in humans. We examined maternal IL-6, measured in each trimester of pregnancy, as a predictor of functional brain network strength in neonates (N=58, M=26.3 days, SD=13.2 days). We focused on the default mode network (DMN) as it appears to be influenced by events prior to term gestational age (e.g. preterm birth), and is associated with mental health status in children and adults. Higher average gestational maternal IL-6 was associated with weaker DMN connectivity involving the posterior cingulate cortex (PCC), dorsal and subgenual medial prefrontal cortex (MPFC), and bilateral lateral parietal cortex. Especially during the first trimester of pregnancy, higher maternal IL-6 was associated

with weaker newborn PCC to MPFC connectivity. This research provides support for the role of maternal gestational immune activation in offspring risk for psychiatric disease via effects on functional brain organization particularly during the first trimester of pregnancy.

Disclosures: **C. Buss:** None. **A.M. Graham:** None. **M.D. Rudolph:** None. **J. Rasmussen:** None. **S. Entringer:** None. **P.D. Wadhwa:** None. **D.A. Fair:** None.

Nanosymposium

584. Early Exposure to Stress: Environmental Factors

Location: 147B

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 584.03

Topic: E.05. Stress and the Brain

Support: Diabetes Australia Research Trust

Title: High fat/sugar diet restored heightened anxiety-like behaviour induced by early life stress and paradoxically reduced hippocampal neurotrophic and mitochondrial biogenesis genes in male rats

Authors: ***J. MANIAM**, C. ANTONIADIS, M. J. MORRIS;
Pharmacol., UNSW Australia, Sydney, Australia

Abstract: Childhood maltreatment including neglect is an example of early life stress, and its incidence is reported to be drastically increasing across the globe. Childhood maltreatment is a major contributor to several psychological disorders particularly anxiety in later life. One common feature of childhood maltreatment is lack of maternal care. We modelled early life stress using limited nesting material (LN) in rats. This model resembles a human condition where the mother is present but care is fragmented (Ivy et al., 2008). As humans commonly binge on palatable diet as a strategy to cope with stress, we examined if palatable high fat/sugar diet (HFHS) would reverse the heightened anxiety-like behavior induced by early life stress. Sprague Dawley rat pups and their dams were provided with a metal grid, a half paper towel and no bedding from postnatal days 2-9, thereby creating a harsh early environment for the pups and dams. Our data revealed that LN resulted in increased anxiety-like behavior at 7 weeks of age as assessed with elevated plus maze. LN pups exhibited a lower percentage of entries into the open arm versus control pups (15.62±1.92 versus 24.98±2.37). Interestingly, LN pups consuming HFHS exhibited reduced anxiety-like behavior as these rats almost doubled the entries into open arms versus LN pups consuming chow (30.50±3.06 versus 15.60±1.91, $p<0.01$). While the

HFHS restored anxiety-like behavior induced by LN, it produced long-term suppression of expression in genes related to neurogenesis and mitochondrial biogenesis in those rats exposed to LN versus unstressed control rats consuming the same diet (table 1). This study reveals for the first time that consumption of a poor diet during adulthood following early life stress increases the risk of developing neuropsychiatric diseases and is associated with deficits in hippocampal plasticity and mitochondrial biogenesis. Reference Ivy, A.S., Brunson, K.L., et al. (2008). Dysfunctional nurturing behavior in rat dams with limited access to nesting material: A clinically relevant model for early-life stress. Neuroscience 154, 1132-42.

Table 1: HFHS feeding following LN decreases expression of hippocampal genes

	Control + Chow	Control + HFHS	LN + Chow	LN + HFHS
Reelin	1.04 ±0.11	1.41±0.10	0.80±0.05	0.65±0.03†††
Neuritin	0.90±0.05	1.01±0.08	0.86±0.10	0.69±0.05††
Nuclear respiration factor 1	1.01±0.04	1.01±0.10	0.75±0.08†	0.62±0.05†††
Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha	1.02±0.07	1.15±0.12	1.13±0.17	0.80±0.05†
†p				

Disclosures: **J. Maniam:** None. **C. Antoniadis:** None. **M.J. Morris:** None.

Nanosymposium

584. Early Exposure to Stress: Environmental Factors

Location: 147B

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 584.04

Topic: F.02. Animal Cognition and Behavior

Support: KAKENHI #23500474

Intramural Research Grant for Neurological and Psychiatric Disorders of the National Center of Neurology and Psychiatry, #22-5, #24-2, and #25-1

Grant-in-Aid for Scientific Research on Innovative Areas, Foundation of synapse and neurocircuit pathology

Title: Modulation of fear memory by dietary polyunsaturated fatty acids via cannabinoid receptors

Authors: *D. YAMADA[1], J. TAKEO[2], P. KOPPENSTEINER[1], K. WADA[1], M. SEKIGUCHI[1]; [1]Natl. Inst. of Neurosci., Kodaira, Japan; [2]Central Res. Laboratory, Nippon Suisan Kaisha, Hachioji, Japan

Abstract: Although the underlying mechanism remains unknown, several studies have suggested benefits of n-3 long-chain polyunsaturated fatty acid (PUFA) for patients with anxiety disorders. Elevated fear is thought to contribute to the pathogenesis of particular anxiety disorders. The aim of the present study was to evaluate whether the dietary n-3 to n-6 PUFA (3:6) ratio influences fear memory. For this purpose, the effects of various dietary 3:6 ratios on fear memory were examined in mice using contextual fear conditioning, and the effects of these diets on central synaptic transmission were examined to elucidate the mechanism of action of PUFA. We found that fear memory correlated negatively with dietary, serum, and brain 3:6 ratios in mice. The low fear memory in mice fed a high 3:6 ratio diet was increased by the cannabinoid CB1 receptor antagonist rimonabant, reaching a level seen in mice fed a low 3:6 ratio diet. The agonist sensitivity of CB1 receptor was enhanced in the basolateral nucleus of the amygdala (BLA) of mice fed a high 3:6 ratio diet, compared with that of mice fed a low 3:6 ratio diet. Similar enhancement was induced by pharmacological expulsion of cholesterol in the neuronal membrane of brain slices from mice fed a low 3:6 ratio diet. CB1 receptor-mediated short-term synaptic plasticity was facilitated in pyramidal neurons of the BLA in mice fed a high 3:6 ratio diet. These results suggest that the ratio of n-3 to n-6 PUFA is a factor regulating fear memory via cannabinoid CB1 receptors.

Disclosures: D. Yamada: None. **J. Takeo:** None. **P. Koppensteiner:** None. **K. Wada:** None. **M. Sekiguchi:** None.

Nanosymposium

584. Early Exposure to Stress: Environmental Factors

Location: 147B

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 584.05

Topic: E.05. Stress and the Brain

Support: ARC Grant DP120104925

ARC Grant DP0985554

Petre Foundation Scholarship

UNSW Research Excellence Award

Title: Foods of the father: Probiotics save infant rats from the transgenerational effects of early stress on fear-related behaviours

Authors: *C. S. COWAN, B. L. CALLAGHAN, R. RICHARDSON;
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Abstract: The effects of early life stress (ELS) on mental and physical health are pervasive and persistent. Indeed, the consequences of ELS have been demonstrated to extend across multiple generations; the offspring of individuals exposed to ELS are vulnerable to the same alterations in emotional and physical function as their parents, even in the absence of direct exposure to ELS. In the current series of experiments, we demonstrate that maternal separation (MS; a rodent model of ELS) alters the developmental trajectory of fear extinction behaviours in two generations of male rats. Further, we show these effects are attenuated by treatment with the probiotic compound Lacidofil®. In Experiment 1, the first generation (F0) of animals were either standard-reared (SR) or exposed to MS from P2-14. For Experiment 2, some of these SR and MS males were then bred in adulthood to produce the second generation (F1) of rats, none of which were exposed to MS stress. In both generations, SR infants exhibited relapse-resistant extinction, but MSF0 and MSF1 infants were prone to relapse following extinction. In Experiment 3, lactating dams received either probiotic or vehicle in their drinking water for the duration of MS (i.e., P2-14). As in the previous experiments, vehicle-treated MS infants exhibited fear relapse following extinction. However, MS infants in the probiotic treatment group did not, exhibiting age-appropriate resistance to relapse similar to unstressed rats. Finally, preliminary data from Experiment 4 suggest that probiotic treatment may also reduce expression of fear relapse in MSF1 rats. Our findings add to the growing body of research highlighting the importance of the brain-gut axis for mental health and emotional development. Further, they provide early support for the use of probiotics to aid treatment of individuals affected by ELS and protect against the consequences of ELS for future generations.

Disclosures: **C.S. Cowan:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Institut Lallemand-Rosell. **B.L. Callaghan:** None. **R. Richardson:** None.

Nanosymposium

584. Early Exposure to Stress: Environmental Factors

Location: 147B

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 584.06

Topic: E.05. Stress and the Brain

Support: Young Researcher Fellowship 2011-2012 of Italian Ministry of Health (GR-2011-02352187)

Title: The transgenerational inheritance of metabolism-related cognitive impairment: epigenetic modifications linking diet and brain health

Authors: *S. FUSCO, A. MASTRODONATO, S. COCCO, C. RIPOLI, S. A. BARBATI, M. SPINELLI, C. GRASSI;
Inst. of Human Physiol., Univ. Cattolica Del Sacro Cuore, Rome, Italy

Abstract: Background and objective The central nervous system (CNS) undergoes structural and functional changes throughout adulthood in response to environmental conditions. Overnutrition alters normal cell signaling in the brain, potentially interfering with both synaptic function and adult neurogenesis, thereby leading to reduced “mindspan” (the maintenance of mental abilities throughout life) and increased risk of neurodegenerative disorders. 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 investigated: 1) how metabolic stress during the early stages of CNS development affects cognitive performance in adulthood; 2) whether the metabolism-dependent functional changes are transmitted transgenerationally via epigenetic mechanisms. Methods 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 offspring (first generation, F1) of both SD- and HFD-fed mice, hereinafter referred as F1-SD and F1-HFD, and their descendants (F2-SD and F2-HFD; F3-SD and F3-HFD, respectively) were all fed with SD and their cognitive performances were evaluated by behavioral and electrophysiological tests. Results Our findings demonstrate that maternal overnutrition alters learning and memory in the offspring. In a standard novel object recognition paradigm the F1-HFD mice showed a significantly lower preference for the novel object than the SD mice. Moreover, F1-HFD mice showed significant impairment of spatial learning and memory evaluated with the Morris water maze. Nutrient excess may alter brain health and plasticity at different levels including impairment of synaptic function and/or adult neurogenesis. Electrophysiological analyses on hippocampal brain slices of F1-HFD mice revealed significant deficits of long-term potentiation (LTP) at CA3-CA1 synapses. Finally, behavioral and

functional alterations may arise from changes in gene expression. Maternal HFD reduced multiple BDNF transcripts expression via specific epigenetic mechanisms involving BDNF regulatory sequences. Recent evidence suggests that metabolic dysfunctions are inherited until the third generation via paternal transmission. Similarly to F1, descendants of HFD-fed F0 mice (F2-HFD and F3-HFD) showed reduced LTP at CA3-CA1 synapses and impaired learning and spatial memory. Conclusions Maternal HFD influences BDNF exons expression in the hippocampus of the offsprings and induces trans-generational effects on cognitive function.

Disclosures: **S. Fusco:** None. **A. Mastrodonato:** None. **S. Cocco:** None. **C. Ripoli:** None. **S.A. Barbati:** None. **M. Spinelli:** None. **C. Grassi:** None.

Nanosymposium

584. Early Exposure to Stress: Environmental Factors

Location: 147B

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 584.07

Topic: E.05. Stress and the Brain

Title: Germline and maternal pathways in the transmission of paternal food restriction stress

Authors: ***R. MASHOODH**, I. B. HABRYLO, K. GUDSNUK, F. A. CHAMPAGNE; Psychology, Columbia Univ., New York, NY

Abstract: Paternal environmental experiences can predict developmental outcomes in subsequent generations of offspring. These effects occur even in the absence of paternal care, suggesting that environmentally-induced epigenetic changes within the paternal germline are inherited. Though epigenetic marks may be associated with paternal transmission, the interplay between paternal effects and maternal influences on offspring development may be an important consideration the emergence of complex phenotypes. In the present study we explored the effect of chronic paternal food restriction (FR) in C57BL/6 mice on offspring behavioral development. Offspring were generated through natural mating (NM) and through embryo transfer (ET) to eliminate maternal effects, and reveal the direct influence of the paternal germline. We demonstrate that under NM conditions, females that mate with FR males gain significantly more weight across gestation and show increased levels of postpartum nursing towards their offspring compared to females mated with control-fed (CF) males. This change in maternal investment did not occur in females who served as surrogates that carried embryos derived from the sperm of FR versus CF males, suggesting that male-female interactions at mating are critical for the expression of paternally-induced maternal effects. Further, we show that offspring phenotype is

dependent on whether offspring were generated through NM or ET. For example, while offspring of FR fathers who were derived through ET show reduced growth rates, memory impairments and increased depression-like behavior, many of these phenotypes were absent in FR offspring generated through NM. We propose that though some paternal FR effects may be generated though germline transmission, paternal FR can influence prenatal and postnatal maternal investment in offspring. Further, these maternal effects may buffer against paternal the effects of paternal FR stress.

Disclosures: R. Mashoodh: None. **I.B. Habrylo:** None. **K. Gudsnuk:** None. **F.A. Champagne:** None.

Nanosymposium

584. Early Exposure to Stress: Environmental Factors

Location: 147B

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 584.08

Topic: E.05. Stress and the Brain

Support: AIHS 200700595

CIHR 102652

CREATE 371155

NSERC 327364-06

NSERC 298194-20009

Title: Transgenerational and multigenerational programming of altered stress response and mental health

Authors: *J. K. MCCREARY[1,2], Z. T. ERICKSON[2], G. A. S. METZ[2];
[1]Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada; [2]Canadian Ctr. for Behavioural Neurosci., Lethbridge, AB, Canada

Abstract: Early life stress may program lifelong vulnerability to mental illness. We have previously shown in rats that developmental programming by prenatal stress propagates across three generations of the maternal lineage, and increases the risk of preterm birth and poor maternal and offspring health outcomes. Here we hypothesized that if a stressful maternal

environment persists across several generations, the recurrent prenatal stress exposure in each generation may cumulatively enhance stress sensitivity and the susceptibility to mental health impairment. We investigated in rats if (1) multigenerational prenatal stress recurring in three consecutive generations will cumulatively program stress sensitivity and mental health; (2) if effects differ between trans- and multigenerational stress exposure; and (3) if behavioural and epigenetic signatures related to impaired mental health can be reversed by environmental intervention. Dams of the parental F0 generation were socially isolated, which represents a model of moderate stress, from postnatal day 90 until parturition. Their pregnant daughters (F1) and grand-daughters (F2) were either stressed (i.e., multigenerational stress) or remained as non-stressed controls (i.e., transgenerational stress with stress limited to F0 dams). A non-stress family line was used for comparison. In the adult male F3 offspring, anxiety-like behaviors, cognitive function and fine motor skills were measured. Multigenerational stress resulted in an anxious phenotype, hyperactivity, and impaired fine motor function along with altered morphology of cortical neurons. Notably, similar alterations also occurred in the transgenerationally stressed F3 generation, although these animals were not directly exposed to stress in utero. In an effort to reverse the consequences of behavioural and epigenetic programming by stress, we exposed animals to enriched environment (EE) from postnatal days 35-180. EE in all groups improved learning and memory in the water maze and promoted fine motor skills and limb coordination. EE drastically reduced the response to stress across all groups, reflecting improved stress resilience. Follow-up studies focus on the identification of stress-sensitive epigenetic pathways that involve microRNAs related to mental health and stress response. This work should identify mechanisms that mediate stress vulnerability and resilience with downstream effects on endocrine, metabolic and behavioural manifestations in a stress-sensitive phenotype, with possible implications for the discovery of new therapeutic targets or predictive biomarkers of mental health.

Disclosures: **J.K. McCreary:** None. **Z.T. Erickson:** None. **G.A.S. Metz:** None.

Nanosymposium

584. Early Exposure to Stress: Environmental Factors

Location: 147B

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 584.09

Topic: E.05. Stress and the Brain

Support: IOS-1253188

Title: Neonatal injury has sex-specific effects on social play and brain vasopressin receptor binding

Authors: *B. M. COOKE, A. P. ROSS, N. C. VICTORIA, A. HAMKI, A. Z. MURPHY; Neurosci. Inst., Georgia State Univ., ATLANTA, GA

Abstract: Early adverse experience has long-lasting, deleterious effects that can be sex-specific. For example, while neonatal inflammation permanently increases pain thresholds in both sexes, the effects are significantly exacerbated in females. Here, we ask whether neonatal inflammation influences social play and the intensity of forebrain vasopressin receptor binding in a sex-specific manner. Day-old rats were handled or received intraplantar injection of carrageenan (CGN) into their hindpaw, causing local inflammation for 48 - 72 hours. Half of the rats simultaneously received saline vehicle or morphine sulfate at the time of CGN and again 5 hr post-CGN, during the peak of inflammation. After weaning, pups were video recorded in their home cages for 20 min every day for 10 days, and the number of play bouts was counted in each cage. At 35 days of age, brains were processed to visualize the type 1a vasopressin receptor (V1aR) with competitive ligand binding autoradiography in four cortical areas. Morphine alone, in the absence of CGN, led to a profound reduction in social play, whereas CGN alone led to a dramatic increase in the frequency of social play. Both effects occurred specifically in females. Moreover, CGN reduced V1aR binding in the orbitofrontal and piriform cortices irrespective of morphine treatment, and did so specifically in females. Thus, we report three heretofore-unknown sex differences: In females but not males, CGN and morphine treatment have opposing effects on social play, and CGN decreases brain V1aR binding. Unrelieved pain during neonatal medical procedures is commonplace. These results suggest that children too may display effects of such experience in their social behavior that, in turn, may have lasting effects on their psychosocial development.

Disclosures: B.M. Cooke: None. **A.P. Ross:** None. **N.C. Victoria:** None. **A. Hamki:** None. **A.Z. Murphy:** None.

Nanosymposium

584. Early Exposure to Stress: Environmental Factors

Location: 147B

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 584.10

Topic: E.05. Stress and the Brain

Title: Precipitated withdrawal in newborn rats is worse after a 3 day exposure to dexmedetomidine (DEX) vs. clonidine (CLON)

Authors: K. KESAVAN[1], A. MASON[2], R. CHAVEZ-VALDEZ[2], T. EZELL[2], Z. MASTER[2], S. KUDCHADKAR[2], G. MCLEMORE[3], *E. B. GAUDA[2];
[1]Pediatrics/Neonatology, Univ. Of California, Los Angeles, CA; [2]Dept Ped, Johns Hopkins Inst., BALTIMORE, MD; [3]Morgan State Univ., Baltimore, MD

Abstract: Background: Neonates are exposed to sedatives during a critical period of neuronal maturation. Alpha-2 adrenergic receptor agonists (α2RA) such as DEX and CLON provide sedation, decrease need for opiates/benzodiazepines and thus decrease respiratory depression. DEX, a more specific α2RA, has a more rapid onset and a shorter duration of action than CLON. Despite the lack of safety and dosing data [Su F et al.], DEX use is increasing in pediatric/neonatal intensive care units. DEX was thought not to cause dependence, but in our clinical experience, similar to published reports [Carney et al., Wadia R et al.] infants develop tolerance and can have protracted withdrawal after exposure to DEX for ≥48 hrs. Here we characterize the behavioral and biochemical withdrawal profile after short exposure to DEX and CLON in a newborn model of precipitated withdrawal. Hypothesis: Precipitated behavioral withdrawal will be more severe and associated with higher levels of brain cAMP in DEX vs. CLON pups after 3 days of continuous exposure. Design/Methods: Osmotic pumps were inserted subcutaneously in Sprague Dawley rat pups at p10-11 to deliver saline (SAL), DEX (2.5 μg/kg/day) or CLON (75 μg/kg/day, loading 20 μg/kg/day). After 3 days, a selective α2R antagonist, atipamezole (ATI) was injected intraperitoneally (0.5mg/kg) to precipitate withdrawal. For 10 minutes after ATI, withdrawal symptoms [tremors (TRM), wet dog shakes (WDS), & jumps (JPS)] were observed. Thereafter, locus coeruleus (LC), periventricular grey matter (PVG) and thalamus (THAL) were removed and processed for cAMP. [n=10/group] Results: DEX induces severe withdrawal symptoms following a precipitated withdrawal after a 3 day exposure characterized by a trend towards more WDS ($p=0.085$) and significantly higher TRM ($p<0.0001$, ANOVA, Tukey post-hoc test) and JPS ($p=0.007$, ANOVA) compared to SAL controls. In contrast CLON exposed pups had only significantly more TRM ($p<0.0001$ vs. SAL), but no difference in the WDS or JPS. DEX exposure is associated with higher combined withdrawal score (CWS) with a median CWS of 2.5 (IQR 1-4) vs. SAL (*$p=0.002$, ANOVA). In contrast, CLON CWS was not different from SAL. DEX exposed animals have twice as much cAMP accumulated in the THAL when compared to SAL controls at 10 minutes after administration of ATI ($p=0.032$, Kruskal Wallis ANOVA, n=4). In contrast cAMP levels in CLON exposed animals was not different from SAL controls. Conclusions: Similar to previous reports in human infants, 3 days of continuous exposure to DEX causes severe withdrawal in a newborn rat model of precipitated withdrawal. Super-activation of cAMP pathways in the THAL is associated with these behavioral findings.

Disclosures: K. Kesavan: None. **E.B. Gauda:** None. **A. Mason:** None. **T. Ezell:** None. **Z. Master:** None. **R. Chavez-Valdez:** None. **S. Kudchadkar:** None. **G. McLemore:** None.

Nanosymposium

584. Early Exposure to Stress: Environmental Factors

Location: 147B

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 584.11

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant MH093362

Title: Microglial inflammatory responses in CO2 evoked fear

Authors: *L. L. VOLLMER[1], S. N. SCHMELTZER[1], I. LEWKOWICH[2], R. W. PUTNAM[3], R. SAH[1];
[1]Psychiatry and Behavioral Neurosci., Univ. Of Cincinnati, Cincinnati, OH; [2]Cell. and Mol. Immunol., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [3]Neuroscience, Cell Biology, and Physiol., Wright State Univ., Dayton, OH

Abstract: Fear can be evoked by both external psychogenic threats and internal homeostatic triggers. Increased carbon dioxide (CO2) concentration, hypercarbia, is a potent homeostatic danger signal that evokes intense fear and panic attacks in susceptible individuals. The molecular identity of CO2 chemosensers and their association with fear and panic-associated responses remains unclear. Previously, we characterized the expression of an acid sensing G-protein coupled receptor, the T-cell death associated gene-8 (TDAG8) receptor, to microglial cells abundant in sensory circumventricular organs (CVOs). Microglia, innate immune cells of the central nervous system (CNS) are recruited in the initiation of pathological responses to homeostatic imbalance. Microglia can transform rapidly from a resting to a pro-inflammatory activated state upon sensing subtle imbalance in ionic homeostasis. Using a 5% CO2 inhalation challenge, we observed significant microglial activation within the subfornical organ (SFO) that was TDAG8 dependent. Minocycline, a microglial activation blocker, significantly attenuated fear responses to CO2 inhalation, accompanied by a significant reduction of microglial activation within the SFO. Current studies are investigating the link between microglial activation and CO2 responses using in vitro and in vivo approaches. Proinflammatory cytokines are released by microglia in response to physiological insults. Measurement of pro-inflammatory cytokines IL-1β, IL-6, and TNF-α revealed a SFO-selective reduction of IL-1β in TDAG8-/- mice that elicit attenuated CO2 evoked fear. Administration of active IL-1β to minocycline-treated mice restored freezing, suggestive of its role as a primary effector in CO2 evoked fear. Using primary microglia cultures from TDAG8-/- and TDAG8+/+ mice and SFO slice electrophysiology we are further investigating the underlying mechanism of IL-1β release and subsequent neuronal activation. Collectively, our data underscore the relevance of microglial acid sensing and

associated proinflammatory responses in CO2-evoked fear. Our novel findings identify microglia as unique chemosensory cells for detecting and translating hypercarbia to fear, providing a novel basis for understanding panic pathophysiology. Support from R01 MH093362

Disclosures: **L.L. Vollmer:** None. **S.N. Schmeltzer:** None. **R. Sah:** None. **I. Lewkowich:** None. **R.W. Putnam:** None.

Nanosymposium

585. Brain Glucose and Energy-Sensing

Location: 152B

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 585.01

Topic: E.07. Food Intake and Energy Balance

Support: FRQS Réseau CMDO

CIHR

Societé Francophone du Diabète

Title: Role of acyl-coa binding protein (acbp) in hypothalamic control of energy balance

Authors: L. BUDRY[1], K. BOUYAKDAN[1], B. TAIB[1], C. CHRETIEN[2], D. RODAROS[1], F. LIENARD[2], A.-B. MARCHER[3], D. NEESS[3], S. MANDRUP[3], L. PENICAUD[2], N. J. FAERGEMAN[3], X. FIORAMONTI[2], *T. ALQUIER[1];
[1]CRCHUM, Montreal, QC, Canada; [2]Univ. de Bourgogne, Dijon, France; [3]Univ. of Southern Denmark, Odense, Denmark

Abstract: Hypothalamic controls of energy balance rely on the detection of circulating nutrients such as glucose and long-chain fatty acids (LCFA) by the medio-basal hypothalamus (MBH). LCFA act in the MBH to inhibit food intake and glucose production. While LCFA intracellular metabolism is required for hypothalamic LCFA sensing and action, the metabolic pathways and cell types involved remain elusive. Acyl-CoA Binding Protein (ACBP) binds intracellular LCFA-CoA with high affinity and regulates their intracellular metabolism in peripheral tissues. In the brain, ACBP is also known as Diazepam Binding Inhibitor, a peptide secreted by astrocytes and cleaved to generate the octadecaneuropeptide (ODN). Central administration of DBI or ODN inhibits the binding of benzodiazepine on the GABAA receptor and exerts anxiogenic and anorectic action. However, the role of the endogenous peptide in hypothalamic LCFA metabolism, sensing and control of energy balance has not been studied yet. We tested if

ACBP is involved in hypothalamic LCFA sensing and energy balance by acting as a regulator of LCFA metabolism and a gliotransmitter targeting anorectic neurons. First, we show that ACBP is mainly expressed in astrocytes and tanycytes in the MBH. Second, intracellular metabolism of oleate but not palmitate is altered in cultured hypothalamic astrocytes and explants derived from ACBP null mouse. Third, using electrophysiological recordings on brain slices from POMC-GFP mice, we found that ODN decreases POMC neurons inhibitory postsynaptic currents frequency and increases their firing rate. This effect was specific to POMC neurons since the firing rate of non-POMC neurons was not affected by ODN. Together, these results support a dual role for glial ACBP as a regulator of LCFA metabolism and a gliotransmitter activating anorectic POMC neurons in the MBH. To test the role of glial ACBP on energy balance, we generated an astrocyte-specific ACBP KO mouse using the Cre-Lox strategy (ACBPGFAP KO). Astrocyte-specific deletion of ACBP does not affect anxiety, food intake, satiety, weight gain or glucose tolerance in male mice fed with a regular chow or high fat diet (HFD). Female mice however show increased weight gain and fat mass when fed with HFD without changes in food intake suggesting that ACBP deficiency in astrocytes increases the susceptibility to diet-induced obesity in a gender-specific manner. Experiments assessing energy expenditure and response to central LCFA in ACBPGFAP KO mice are underway. Collectively, these data suggest the existence of an ACBP-dependent cross-talk between astrocytes and neurons involved in LCFA sensing and hypothalamic regulation of energy balance.

Disclosures: **L. Budry:** None. **K. Bouyakdan:** None. **B. Taib:** None. **C. Chretien:** None. **D. Rodaros:** None. **F. Lienard:** None. **A. Marcher:** None. **D. Neess:** None. **S. Mandrup:** None. **L. Penicaud:** None. **N.J. Faergeman:** None. **X. Fioramonti:** None. **T. Alquier:** None.

Nanosymposium

585. Brain Glucose and Energy-Sensing

Location: 152B

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 585.02

Topic: E.07. Food Intake and Energy Balance

Support: CIG NeuROSens

SFD/ResMed

INRA, CNRS, Université de Bourgogne

Région Bourgogne

Title: Glucose excites hypothalamic neurons through the activation of Transient Receptor Potential Canonical (TRPC) channels

Authors: C. CHRÉTIEN, C. FENECH, S. GRALL, L. PÉNICAUD, C. LELOUP, *X. FIORAMONTI;
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Abstract: The mediobasal hypothalamus (MBH) houses specific glucose-sensitive neurons able to sense changes in glucose levels which are suggested to participate in the control of glucose homeostasis. High-glucose excited (HGE) neurons increase their electrical activity in response to increased glucose level. Despite the involvement of non-selective cationic channels (NSCCs), molecular mechanisms involved in HGE neuron glucose response are unknown. Mitochondrial Reactive Oxygen Species (mROS) are produced into the MBH in response to increased glucose and involved in glucose detection. Interestingly, some transient receptor potential canonical (TRPC) channels are NSCCs directly modulated by ROS. Here, we hypothesized that HGE neuron detect increased glucose level through a ROS-TRPC dependent signaling pathway. To test this hypothesis, dissociated rat MBH cells activity in response to increased glucose, was monitored using Fura-2 calcium imaging in presence of TRPC channel inhibitors or antioxidants. Hypothalamic detection of hyperglycemia was also evaluated *in vivo* by measuring insulin secretion in response to an intra-carotid glucose load. We first investigated the presence of the redox-sensitive TRPC3/4 channels in the MBH and directly into HGE neurons by classic or single-cell RT-PCR, respectively. Quantification of the area under the curve (AUC) of HGE neurons glucose responses shows that ~100% of MBH HGE neuron responses to 2.5-10 mM increased glucose are inhibited by antioxidants (trolox + gluthation [79.4 ± 7.4 % inhibition; $p < 0.05$] or catalase [82.4 ± 10.9 % inhibition; $p < 0.05$]) or the non-selective TRPC channel inhibitor SKF96365 (89.3 ± 4 % inhibition; $p < 0.05$). Glucose responses are partially inhibited by the TRPC3 or TRPC4 channel inhibitors Pyr3 (69 ± 12.5 % inhibition; $p < 0.05$) and ML204 (89.4 ± 4.9 % inhibition; $p < 0.05$) or mimicked by the TRPC3 activator OAG (98.3 ± 34.7 % activation *vs* 10 mM glucose; $p > 0.05$), respectively in ~70% of HGE neurons. Interestingly, simultaneous inhibition of TRPC3/4 channels blocks glucose responses by 97.2 ± 1.71 % ($p < 0.05$) in 82% of HGE neurons. *In vivo*, pharmacological inhibition of TRPC3 channel specifically into rat MBH significantly decreases insulin secretion by 42.1 ± 11 % ($p < 0.05$) in response to intra-carotid glucose injection. Finally, preliminary data show that hypothalamic detection of increased blood glucose level is also impaired in TRPC3 deficient mice where the presence of MBH HGE neurons is being explored. Altogether, these data highlight a new ROS-TRPC3/4 channel dependent pathway involved in HGE neuron glucose response and the central control of glucose homeostasis.

Disclosures: C. Chrétien: None. **X. Fioramonti:** None. **C. Fenech:** None. **S. Grall:** None. **L. Pénicaud:** None. **C. Leloup:** None.

Nanosymposium

585. Brain Glucose and Energy-Sensing

Location: 152B

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 585.03

Topic: E.07. Food Intake and Energy Balance

Support: Supported by CIHR

Title: The monounsaturated fatty acid oleate in the ventral tegmental area inhibits feeding behaviour and dopamine neurotransmission

Authors: C. HRYHORCZUK[1,2,3], Z. SHENG[4], V. ROUTH[4], T. ALQUIER[1,2,3], *S. E. FULTON[1,2,3];
[1]CRCHUM, Montreal, QC, Canada; [2]Univ. de Montréal, Montreal, QC, Canada; [3]Montreal Diabetes Res. Ctr., Montreal, QC, Canada; [4]Rutgers, New Brunswick, NJ

Abstract: Dopamine (DA) neurons of the ventral tegmental area (VTA) are critical for the control of motivation and reward-relevant behaviors. Evidence that DA neurons respond to hormones like leptin, ghrelin and insulin to modulate feeding, reward-relevant behavior and DA tone raises the possibility that, as in the hypothalamus, cells of the VTA act as metabolic sensors that integrate both hormonal and nutrient signals. The aim of the present work was to evaluate the impact of long-chain fatty acids (FA) in the VTA on feeding and DA neurotransmission. Methods: Following stereotaxic implantation of a double cannula into the VTA, male Wistar rats (n=12-15/group) received either vehicle (2-HydroxyPropyl-β-cyclodextrin (HPB) in ACSF; 500nl), oleate (12mM; monounsaturated FA) or palmitate (12mM; saturated FA). Patch clamp recordings were made in rat VTA slices preparations perifused with oleate (6µM, 2.5mM glucose; n=15) or oleate+phloretin (6µM; 2.5mM glucose; 100µM phloretin; n=6), to block fatty acid transport. Results and conclusion: Behavioral results show that a single injection of oleate, but not palmitate, in the VTA significantly decreased dark cycle chow intake. Oleate significantly inhibited firing in ~50% of DA neurons recorded - an effect blocked by phloretin, and reduced the amplitude but not the frequency of mEPSCs. Together, the findings suggest that oleate in the VTA has anorectic actions that may involve intracellular transport of FA and inhibition of DA neurotransmission and offer a means whereby dietary FA may directly modulate brain reward circuitry.

Disclosures: C. Hryhorczuk: None. **S.E. Fulton:** None. **T. Alquier:** None. **Z. Sheng:** None. **V. Routh:** None.

Nanosymposium

585. Brain Glucose and Energy-Sensing

Location: 152B

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 585.04

Topic: E.07. Food Intake and Energy Balance

Support: CIHR grant MOP-102511

Title: Glia-neuron interaction and energy homeostasis

Authors: *M. V. KOKOEVA, T. DJOGO, S. ROBINS, D. KRYZSKAYA, T. STROH;
Med., McGill Univ., Montreal, QC, Canada

Abstract: We have previously shown that the adult hypothalamus continuously produces new cells even in the absence of exogenous stimuli. In a search for the proliferation source, we further discovered that the majority of adult-born hypothalamic cells are generated by oligodendrocyte precursor cells (OPCs), which appear to act as hypothalamic stem cells as they mature into oligodendrocytes, but also give rise to neuronal marker-expressing cells that exhibit electrophysiological features of bona fide neurons. Here we provide new evidence for a role of glia cells in energy balance. We show that specific glia contribute to body weight maintenance by affecting leptin sensing.

Disclosures: M.V. Kokoeva: None. **T. Djogo:** None. **S. Robins:** None. **D. Kryzskaya:** None. **T. Stroh:** None.

Nanosymposium

585. Brain Glucose and Energy-Sensing

Location: 152B

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 585.05

Topic: E.07. Food Intake and Energy Balance

Support: NIH K01DK087780 to KWW

American Diabetes Association 7-11-MN-16 to TL

American Heart Association – 9SDG2080223 to MF

NIH F32 DK092083 and K01 DK098317 to EDB

American Heart Association 13POST16710016 to XK

NIH R01DK53301, R01DK088423, and RL1DK081185 to JKE

NIH R01DK55758 to PES

Title: Xbp1s in Pomc neurons connects ER stress with energy balance and glucose homeostasis

Authors: *K. W. WILLIAMS[1], T. LIU[1], M. FUKUDA[3], Y. DENG[1], X. KONG[4], E. D. BERGLUND[2], Z. DENG[1], J.-W. SOHN[1], M. M. SCOTT[5], S. LEE[1], C. E. LEE[1], P. E. SCHERER[1], J. K. ELMQUIST[1];
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Abstract: Obesity is associated with leptin resistance, while type 2 diabetes is characterized by insulin resistance in multiple tissues. The molecular mechanisms underlying neuronal leptin and insulin resistance in obesity and diabetes remain unclear. Recently, endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) have emerged as a unifying and critical link in the development of cellular leptin and insulin resistance. Here we show that induction of the UPR transcription factor “spliced X-box binding protein 1” (Xbp1s) in pro-opiomelanocortin (Pomc) neurons alone is sufficient to protect against diet-induced obesity as well as improve leptin and insulin sensitivity - even in the presence of strong activators of ER stress. The improved body weight was accompanied by increased energy expenditure and heat production. Constitutive expression of Xbp1s in Pomc neurons contributes to improved hepatic insulin sensitivity and suppression of endogenous glucose production. Together our results identify critical molecular mechanisms linking ER stress in arcuate Pomc neurons to acute leptin and insulin resistance as well as liver metabolism in diet-induced obesity and diabetes.

Disclosures: K.W. Williams: None. **T. Liu:** None. **M. Fukuda:** None. **Y. Deng:** None. **X. Kong:** None. **E.D. Berglund:** None. **Z. Deng:** None. **J. Sohn:** None. **M.M. Scott:** None. **S. Lee:** None. **C.E. Lee:** None. **P.E. Scherer:** None. **J.K. Elmquist:** None.

Nanosymposium

585. Brain Glucose and Energy-Sensing

Location: 152B

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 585.06

Topic: E.07. Food Intake and Energy Balance

Support: ANR Lipobrain

EFSD Lilly Fellowship

CORRDIM

Title: Specific deletion of lipoprotein lipase in ventromedian hypothalamus induces obesity and impairment of glucose homeostasis in mice

Authors: E. LAPERROUSAZ, V. S. MOULLÉ, N. KASSIS, R. G. DENIS, S. LUQUET, C. MAGNAN, *C. CRUCIANI-GUGLIELMACCI;
Univ. Paris Diderot, BFA, Paris, France

Abstract: Brain lipid sensing is a key regulator of nervous control of energy balance. Indeed specific neurons are able to sense change in plasma free fatty acid concentrations and in turn finely control energy homeostasis such as food intake and feeding behavior, energy expenditure as well as insulin secretion and action. In addition it has been demonstrated that triglyceride-enriched particles such as VLDL could be also sensed by specialized neurons. Thus, neuronal lipoprotein lipase (LPL)-depletion leads to obesity (1). Using LPL-floxed mice injected with AAV-cre, we recently evidenced that specific depletion of LPL in hippocampus also leads to obesity as consequence of increased parasympathetic nervous activity and decreased in both locomotor activity and energy expenditure (2). Finally, specific depletion of LPL in accumbens nucleus increased both palatable food preference and food seeking behavior (3). The present study was aimed at testing whether specific depletion of LPL in another key area regulating energy balance, ventromedian hypothalamus (VMH), also deregulated energy balance. To that end, LPL-floxed and WT (controls) mice received a bilateral injection of AAV-cre in VMH and following parameters were measured during 12 weeks: body weight, food intake, energy expenditure, locomotor activity. Both glucose and insulin tolerance tests were also performed to assess glucose homeostasis. LPL activity was decreased by about 30% in AAV-cre injected mice compared to controls. VMH LPL-depleted mice developed obesity as well as basal hyperglycemia, hyperinsulinemia and hyperleptinemia. There was no change in food intake compared to controls but both energy expenditure and locomotor activity were significantly decreased. Finally, these mice became glucose intolerant and insulin resistant. We conclude that specific depletion of LPL in VMH impaired nervous control of energy balance leading to obesity and deregulation of glucose homeostasis thus highlighting LPL as a key actor of brain lipid sensing in hypothalamus. References 1: Wang et al, Cell Metab 2011 2: Picard et al, Mol Metab, 2013 3: Cansell et al, Mol Psy, 2014

Disclosures: **E. Laperrousaz:** None. **V.S. Moullé:** None. **N. Kassis:** None. **R.G. Denis:** None. **S. Luquet:** None. **C. Magnan:** None. **C. Cruciani-Guglielmacci:** None.

Nanosymposium

585. Brain Glucose and Energy-Sensing

Location: 152B

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 585.07

Topic: E.07. Food Intake and Energy Balance

Title: Direct input to orexin/hypocretin neurones from forebrain areas related to metabolism regulation

Authors: ***A. GONZALEZ**, D. BURDAKOV;
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Abstract: Orexin/hypocretin (orx/hcrt) neurones in the lateral hypothalamus are essential for the maintenance of metabolic homeostasis. These cells are modulated by both neural and humoral signals, though the circuitry and mechanisms involved are not yet fully understood. To investigate which brain areas project directly to orx/hcrt cells we injected adeno-associated viruses into the lateral hypothalamus of mice that expressed cre specifically in these neurones. These viruses led in turn to the expression of the glycoprotein RG and the receptor TVA in orx/hcrt cells. A later injection into the same area of a modified rabies virus resulted in the retrograde labelling of only those neurones in the brain that send monosynaptic inputs to orx/hcrt cells. We found that most inputs to orx/hcrt cells originated locally from other hypothalamic regions, mainly from the paraventricular and supraoptic nuclei; the anterior, ventromedial and dorsomedial hypothalamus; and the arcuate hypothalamic nucleus. In addition, numerous extrahypothalamic inputs to orx/hcrt cells were also observed, notably from the bed nucleus of the stria terminalis, the lateral septum, the central amygdala, and the nucleus accumbens. Because the arcuate nucleus is also a well-known component of the circuitry that regulates metabolism homeostasis, we then tested for direct connectivity between orx/hcrt neurones and cells in the arcuate using in vitro electrophysiology and optogenetics. Expression of the light-activated channel channelrhodopsin was directed to only orx/hcrt-cre cells with an adeno-associated virus injected into the lateral hypothalamus. These mice also expressed green-fluorescent protein (GFP) in neuropeptide-Y (NPY) cells, which constitute a major population of neurones in the arcuate. Whole-cell recordings in brain slices were made from GFP-NPY cells in the arcuate during light stimulation of orx/hcrt fibres. We found no evidence of a functional

connection from orx/hcrt in the lateral hypothalamus to NPY cells in the arcuate. Since there was anatomical evidence of a monosynaptic connection in the opposite direction, these observations illustrate a case where there is directionality in the link between two specific nodes in the network that regulates metabolism. It was interesting that most inputs to orx/hcrt neurones originated in brain regions that are known to be involved in modulating several aspects of energy homeostasis. Our findings improve our understanding of the network that controls metabolism in the body.

Disclosures: A. Gonzalez: None. **D. Burdakov:** None.

Nanosymposium

585. Brain Glucose and Energy-Sensing

Location: 152B

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 585.08

Topic: E.07. Food Intake and Energy Balance

Support: NJMS Foundation Grant

Title: Activation of lateral hypothalamic area (LHA) orexin glucose-inhibited (GI) neurons in low glucose induces glutamate plasticity on ventral tegmental area (VTA) dopamine neurons

Authors: *V. H. ROUTH[1], Z. SHENG[1], M. P. THOMAS[2];
[1]Dept Pharmacol & Physiol, RBHS: New Jersey Med. Sch., Newark, NJ; [2]Univ. Northern CO, Greeley, CO

Abstract: Lateral hypothalamic area (LHA) orexin neurons modulate reward-based feeding by activating ventral tegmental area (VTA) dopamine (DA) neurons. We recently found that signals of peripheral energy status influence reward-based feeding by modulating the glucose sensitivity of LHA orexin glucose-inhibited (GI) neurons. Leptin blunts while ghrelin and fasting potentiate the activation of LHA orexin-GI neurons in low glucose. Activation of LHA orexin-GI neurons by decreased glucose is sufficient to increase glutamate-mediated spontaneous excitatory post-synaptic currents (sEPSCs) onto VTA DA neurons. In the present study we hypothesized that VTA orexin release during a longer duration exposure to low glucose could cause persistent alterations in glutamate signaling on VTA DA neurons. This hypothesis was tested using electrophysiological recordings of VTA-DA neurons in a horizontal slice preparation containing the LHA and VTA. Short-term (10 min) glucose decreases increased the frequency of spontaneous excitatory post-synaptic currents (sEPSCs; 125 ± 40%, n = 9, $P<0.05$) and action

potentials (n = 9; P<0.05) in 45% (9/20) of VTA DA neurons. sEPSCs were completely blocked by α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) glutamate receptor antagonists (CNQX 20 μM, n = 4; APV 20 μM, n = 4; respectively), demonstrating that these sEPSCs were mediated by glutamatergic transmission onto VTA DA neurons. A forty minute exposure to 0.25 mM glucose increased the amplitude of NMDA sEPSCs from 12.8 ± 1.1 pA to 27.15 ± 3.4 pA (n = 8; P < 0.05). NMDA current amplitude was still significantly increased 50 -60 min after glucose levels were returned to 2.5 mM (25.0 ± 3.0 pA; n = 8; P < 0.05 vs pre-exposure to low glucose). This suggests that low glucose may lead to glutamate plasticity in VTA DA neurons. When glucose was lowered for 40 min in the presence of the orexin-1 receptor antagonist SB334867 (10 μM; n = 3) glutamate EPSCs were not increased (P>0.05; n = 3). Thus, decreased glucose causes persistent increases in orexin-dependent excitatory glutamate neurotransmission onto VTA DA neurons. These data suggest that the glucose sensitivity of LHA orexin-GI neurons links metabolic state and reward-based feeding.

Disclosures: **V.H. Routh:** None. **Z. Sheng:** None. **M.P. Thomas:** None.

Nanosymposium

585. Brain Glucose and Energy-Sensing

Location: 152B

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 585.09

Topic: E.07. Food Intake and Energy Balance

Support: NIH P20-RR021945

F32-DK097896

R01-DK092587

Title: Preoptic leptin-responsive neurons regulate energy expenditure and body temperature

Authors: ***S. YU**, K. REZAI-ZADEH, H. MÜNZBERG;
Central Leptin Signaling, Pennington Biomed. Res. Ctr., Baton Rouge, LA

Abstract: The adipocyte-derived hormone leptin increases energy expenditure in part by activating central thermogenic pathways. Consistent with this function, the long form leptin receptor (LepRb) is expressed in several key thermoregulatory regions including the preoptic area (POA). Discovery of brown adipose tissue (BAT) in adult humans stirred tremendous

interest in how to activate BAT to increase energy expenditure as a potential treatment for obesity. Thus, it is critical to understand how BAT thermogenesis is regulated. To test if POA LepRb neurons indeed mediate thermoregulatory function, we set out to study their neurochemical phenotypes, synaptic connections, and physiological relevance. Because changes in temperature robustly activate distinct POA neurons, we first tested the thermoregulatory effect of neuronal activation selectively in POA LepRb neurons by viral expression of designer receptor exclusively activated by designer drug (DREADD)-Gq in LepRb-Cre mice. Activation of POA LepRb neurons dramatically decreased the core body temperature and energy expenditure, implying the activation of heat defense mechanisms. Behaviorally, these mice exhibited relaxed postural extension, a typical behavior for mice at thermoneutrality. Consistent with this, POA LepRb neurons were activated by acute (3h) warm (30oC), but not cold (4oC), exposure. Using Vglut2- and Vgat-YFP reporter mice we found that POA LepRb neurons are mostly glutamatergic, contradicting other data suggesting that warm-sensitive POA>DMH/DHA projecting neurons are GABAergic. We further verified that glutamatergic neurons truly mediated the heat defense responses, by viral DREADD-Gq expression and activation of either glutamatergic or GABAergic POA neurons in Vglut2- or Vgat-Cre mice, respectively. Indeed, activation of glutamatergic, but not GABAergic, POA neurons elicited similar heat defense responses as POA LepRb neurons. Surprisingly, activation of POA GABAergic neurons did not modulate body temperature or energy expenditure unless animals were exposed to cold temperature (10oC), where activated POA GABA neurons attenuated cold-induced increases in energy expenditure. This suggests that POA GABA neurons are physiologically relevant only during cold stress. In summary, our data clearly suggest that glutamatergic, not GABAergic, POA neurons drive major heat defense mechanisms and LepRb neurons are essential players during that process. Further studies will investigate if and how leptin itself modulates the activity of POA LepRb neurons and how this contributes to whole body energy homeostasis and body weight regulation.

Disclosures: **S. Yu:** None. **K. Rezai-Zadeh:** None. **H. Münzberg:** None.

Nanosymposium

585. Brain Glucose and Energy-Sensing

Location: 152B

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 585.10

Topic: E.07. Food Intake and Energy Balance

Support: Strategic Research Program for Brain Sciences (10036069) by the Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: Glucose-dependent dual effects of central adiponectin on POMC neurons in arcuate nucleus

Authors: *S. SUYAMA[1], F. MAEKAWA[2], Y. MAEJIMA[1], N. KUBOTA[3], T. KADOWAKI[3], T. YADA[1];
[1]Dept. of Physiology, Div. of Integrativephysiology, Jichi Med. Univ., Tochigi, Japan; [2]Envrn. Hlth. Sci. Division, Ctr. for Envrn. Hlth. Sciences,, Natl. Inst. for Envrn. Studies, Tsukuba, Ibaraki, Japan; [3]Dept. of Diabetes and Metabolic Diseases, Grad. Sch. of Med., Univ. of Tokyo, Tokyo, Japan

Abstract: Adiponectin is known to act on the peripheral tissues and regulate glucose and energy metabolism. It has also been reported that adiponectin enters the brain from circulation and regulates neuronal activities in the hypothalamus and brain stem. Adiponectin receptors, AdipoR1 and R2, are expressed in various brain regions including a feeding center arcuate nucleus (ARC) of hypothalamus where proopiomelanocortin (POMC) neurons function as the first order neurons to induce satiety. To date, the effect of ICV injection of adiponectin on food intake has been studied in several laboratories. However the results are in discrepancy. One report showed that ICV adiponectin injection increased food intake for 6hrs following 3hrs refeeding condition in mice (Kubota et.al., 2007). Another report showed that it decreased food intake in rats (Coope et.al., 2008). These reports used different experimental conditions, including species (mouse or rat), and nutrient status especially glucose. We here show the glucose-dependent dual effects of central adiponectin on food intake. In the present study, adiponectin was ICV injected with glucose to mimic fed conditions, and without glucose to mimic fasted conditions, in mouse. ICV injection of adiponectin with glucose hyperpolarized the membrane of the POMC neurons in ARC slices. In contrast, ICV injection of adiponectin without glucose depolarized the membrane of the ARC POMC neurons. These results indicate that in hunger states with low blood glucose levels (early dark period in mice), central adiponectin promotes the activity of POMC neurons, possibly acting as anorexigenic, whereas in fed states with high blood glucose levels it suppress the POMC neurons activity, possibly acting as orexigenic. Our results provide a clue to settle the discrepancy in previous reports.

Disclosures: S. Suyama: None. **F. Maekawa:** None. **N. Kubota:** None. **T. Kadowaki:** None. **Y. Maejima:** None. **T. Yada:** None.

Nanosymposium

585. Brain Glucose and Energy-Sensing

Location: 152B

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 585.11

Topic: E.07. Food Intake and Energy Balance

Support: NHMRC Grant 1026963

NHMRC Grant 1065237

ARC FT100100966

Rebecca L Cooper Foundation

Title: Carnitine acyltranserfase links a metabolic rhythm to nutirent sensing in the POMC neurons

Authors: *Z. B. ANDREWS[1], G. PREMANATHAN[2], S. H. LOCKIE[2];
[1]Physiol., Monash Uni, Clayton, Australia; [2]Monash Univ., Clayton, Australia

Abstract: Proopiomelanocortin (POMC) neurons are a key component of the hypothalamic melanocortin system, which maintains whole body energy balance. For example, mutations in the melanocortin system lead to obesity in mice and humans. POMC neurons sense nutrients during acute and chronic positive energy balance and engage mechanisms to control energy balance. We reasoned mitochondrial mechanisms regulating glucose metabolism are potentially important metabolic sensors that influence POMC function. Thus, we deleted carnitine acyltransferase (Crat), a mitochondrial matrix enzyme that regulates glucose metabolism and substrate selection, from POMC neurons and examined the effect on energy metabolism. Cre-dependent reporter expression using loxSTOPlox tdTomato mice revealed that greater than >85% of arcuate POMC neurons expressed tdTomato in POMC crat WT and KO mice. High fat diet (HFD) exposure increased body weight gain, fat pad mass and plasma leptin in KO mice relative to WT controls, whereas no difference was observed in mice fed a chow diet. POMC Crat KO mice on HFD diet were more glucose intolerant, although no genotypic differences in plasma insulin were observed during the oral glucose tolerance test. Stereological investigation in WT and KO chow-fed and HFD-fed mice showed no genotypic difference in POMC cell number or volume, indicating deletion of Crat did not affect POMC neuron morphology. We discovered that acute changes in nutrient status, such as fasting/refeeding, peripheral and central glucose injections were dependent upon Crat in POMC neurons. Strikingly, the ability of Crat to sense nutrient feedback was dependent on the time of day. We conducted our experiments at 9am or 9pm, with the latter being the peak time of daily hypothalamic POMC gene expression. We observed that KO mice consumed more food and had attenuated postprandial BAT thermogenic changes relative to WT mice in response to refeeding and acute glucose injections at 9pm. These

changes were not observed at 9am, when mice are metabolic quiescent. Interestingly however, refeeding at 9am elevated food consumption during the subsequent dark phase in KO compared to WT, indicating a long-term failure to adapt to metabolic stimuli. We also used CLAMS and observed Crat-dependent changes in VO2, VCO2 and heat production at 9pm, but not 9am, in response to fasting/refeeding and acute glucose administration. Collectively we have uncovered a novel role for Crat in POMC neurons in energy balance regulation. We believe Crat in an important metabolic sensor of energy acute status that depends on a daily metabolic rhythm in POMC neurons.

Disclosures: Z.B. Andrews: None. **G. Premanathan:** None. **S.H. Lockie:** None.

Nanosymposium

585. Brain Glucose and Energy-Sensing

Location: 152B

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 585.12

Topic: E.07. Food Intake and Energy Balance

Support: Grant n° 31003A-140957 from Fonds National Suisse to LP

SFD 2014 grant to LC

Title: *In vivo* evidence for hypothalamic ketone bodies sensing: impact on food intake and endocrine responses in mice

Authors: *L. CARNEIRO, L. PELLERIN;
UNIL, Lausanne, Switzerland

Abstract: BACKGROUND: Hypothalamic nutrient sensing plays an important role in maintaining energy homeostasis. Moreover, hypothalamic nutrient sensing seems disrupted during obesity development. However, the mechanism underlying such a dysregulation is not completely understood. Interestingly, the MCT1+/- mouse recently developed exhibits a resistance to diet-induced obesity, suggesting that monocarboxylates (via their transport by MCT1 in hypothalamic neurons) might play an important role in the control of energy homeostasis. In this regard, the putative role of ketone bodies has not been thoroughly explored. In this study, we aimed to determine the impact of a rise in cerebral ketone bodies on food intake and energy homeostasis regulation. METHODS: Ketone bodies were infused through the carotid artery during 24h. This infusion route was intended to mimic the physiological transport

occurring from blood to brain parenchyma through the blood brain barrier and relying on the monocarboxylate transporters expressed in these different compartments. At 24h, food intake was measured and concentrations of blood metabolic markers were determined. RESULTS: 24 hours of ketone bodies infusion led to an increase in food ingested. This increase in food intake appeared during the first 6h and lasted until the end of the 24h infusion. The observed stimulated food intake is associated with an increase in mRNA coding for the hypothalamic neuropeptides NPY and AgRP. Finally, increased brain ketone bodies also alters the hormonal profile and leads to an increased insulinemia and altered sensitivity to insulin. Finally, we also unraveled a decrease in glucose production induced by the ketone bodies infusion. CONCLUSION: Altogether, these results suggest that an increase in brain ketone bodies concentration leads to hyperphagia and deregulated energy homeostasis. Moreover, it is purported that the hyperketonemia observed in diet-induced obesity could be at least in part responsible for deregulated metabolic signal sensing and the imbalance in energy homeostasis.

Disclosures: L. Carneiro: None. **L. Pellerin:** None.

Nanosymposium

585. Brain Glucose and Energy-Sensing

Location: 152B

Time: Tuesday, November 18, 2014, 1:00 PM - 4:15 PM

Presentation Number: 585.13

Topic: E.07. Food Intake and Energy Balance

Title: The role of Agouti-related peptide neurons in inflammatory anorexia

Authors: *L. GAUTRON, Y. LIU, Y. HUANG, T. LIU;
Univ. Texas Southwestern Med. Ctr., DALLAS, TX

Abstract: Compelling data demonstrate that inflammation-associated anorexia directly results from the mobilization of a specific neural circuit that reduces appetite. This study investigated the role of Agouti-Related Peptide (AgRP) neurons in inflammatory anorexia caused by the acute administration of lipopolysaccharides (LPS). Specifically, we used designer receptors exclusively activated by designer drugs to manipulate the activity of AgRP neurons during inflammatory anorexia. In AgRP-Cre mice expressing the designer receptor hM4Dq only in AgRP neurons, the administration of the designer drug clozapine-N-oxide induced a robust feeding response lasting for several hours. This feeding response was rapidly and completely suppressed by the concomitant administration of LPS. The above data, combined with additional double-labeling experiments and genetic manipulations of arcuate nucleus neurons, suggest that

AgRP neurons may be crucial mediators of some of the anorectic effects of LPS and reveal novel neural pathways implicated in inflammatory anorexia. A better understanding of the neurobiological mechanisms underlying inflammatory anorexia will help to develop appetite stimulants for anorectic patients.

Disclosures: L. Gautron: None. **Y. Liu:** None. **Y. Huang:** None. **T. Liu:** None.

Nanosymposium

586. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: 140A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 586.01

Topic: F.01. Human Cognition and Behavior

Support: NSF Grant BCS0745880

Title: The nature of true and false memory activity in motion versus shape processing cortex

Authors: *J. M. KARANIAN, S. D. SLOTNICK;
Psychology, Boston Col., Chestnut Hill, MA

Abstract: Previous findings suggest true memory but not false memory can produce activity in earlier visual regions. However, such differential results have only been observed in paradigms that required retrieval of spatial information. The present fMRI study consisted of an experiment on memory for motion, which required spatial retrieval, and an fMRI experiment on memory for shape, which did not require spatial retrieval. Our aim was to determine whether the same or different pattern of true and false memory activity was observed during retrieval of motion or shape information. In the first experiment, moving or stationary shapes were presented at encoding. At retrieval, old shapes were presented and participants classified each shape as previously “moving” or “stationary”. True memory activity was identified by contrasting accurate versus inaccurate memory for moving items (i.e., “moving”/moving > “stationary”/moving, hits > misses). False memory activity was identified by contrasting inaccurate generation of “moving” responses to stationary items versus accurate memory for stationary items (i.e., “moving”/stationary > “stationary”/stationary). In the second experiment, colored abstract shapes that were either intact or scrambled were presented at encoding. At retrieval, colored disks were presented and participants classified the corresponding shape as “intact” or “scrambled”. True memory and false memory contrasts were analogous to those described above (i.e., “intact”/intact > “scrambled”/intact and “intact”/scrambled >

"scrambled"/scrambled, respectively). A random-effect general linear model analysis was conducted with an individual voxel threshold of 0.001, corrected for multiple comparisons to $p < 0.05$. Replicating previous results, true memory but not false memory for motion activated MT+ (even at a reduced threshold of $p < 0.01$). By comparison, a conjunction analysis revealed that true memory and false memory for shape activated LOC. To assess the magnitude of these effects, event-related activation timecourses were extracted from MT+ and LOC. In MT+, true memory for motion produced a significantly greater magnitude of activity than false memory for motion. In LOC, the magnitude of true memory and false memory activity did not significantly differ. Of importance, the condition (true memory, false memory) by region (MT+, LOC) interaction was significant. These results indicate that activity in MT+ and LOC mediates true memory, whereas only activity in LOC mediates false memory. More broadly, these results support the proposal that the "what" pathway and the "where" pathway reflect conscious and nonconscious processing, respectively.

Disclosures: J.M. Karanian: None. **S.D. Slotnick:** None.

Nanosymposium

586. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: 140A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 586.02

Topic: F.01. Human Cognition and Behavior

Support: NSF Grant BCS1025709

Title: Exploring the neural correlates of true and false memory for conceptual pictures in older adults

Authors: *I. C. TURNEY, C. E. JOHNSON, N. A. DENNIS;
The Pennsylvania State Univ., University Park, PA

Abstract: Age differences in false memory have been examined using both semantic and perceptual false memory paradigms, with older adults showing increased neural processing in familiarity or gist processing regions, supporting age-related increases in false memories. While behavioral research has shown false memories can also be elicited stemming from conceptual gist, the neural underpinning of age-related increases in this paradigm have not been examined. The current study utilized a modified scene memory paradigm to investigate neural differences underlying true and false memories elicited from thematic contexts in both younger and older

adults. Results showed that, in both age groups, true memories were associated with increases in neural activity within the typical retrieval network, including left prefrontal, parietal, occipital, and parahippocampal regions. False memories were associated with increases in neural activity within a more limited network including left middle prefrontal cortex and middle and inferior temporal gyrus. Taken together, results suggest that, across age groups, while true retrieval is mediated by retrieval of visual details and general reconstructive processes, false retrieval relies on semantic or categorical processing associated with the thematic context. Directly comparing age groups, we found that older adults exhibited greater activation supporting true memories in the hippocampus and visual cortex - as well as activation in bilateral prefrontal cortex and right middle temporal gyrus. Older adults also exhibited greater activation supporting false memories in middle/superior temporal gyrus, late visual cortex and the prefrontal cortex, suggestive of greater reliance on gist processing. Young adults only exhibited greater activity compared to older adults in a very limited set of regions for both true and false memories. Results will be discussed in terms of differences in neural efficiency between age groups as well as within the framework of aging and the Fuzzy Trace Theory.

Disclosures: I.C. Turney: None. **C.E. Johnson:** None. **N.A. Dennis:** None.

Nanosymposium

586. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: 140A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 586.03

Topic: F.01. Human Cognition and Behavior

Support: NSF Grant BCS1025709

Title: Examining the influence of thematic information on retrieval success

Authors: *C. E. JOHNSON, I. C. TURNEY, N. A. DENNIS;
The Pennsylvania State Univ., University Park, PA

Abstract: Schemas act as memory mechanisms that allow one to build frameworks in order to support memory through the use of gist information. The current study sought to use naturalistic scenes in order to investigate the neural basis of true memories for information that was inherently tied to the scene's schema compared to that which was not related to the schema. During encoding participants viewed thematic scenes (e.g., Christmas, bathroom, camping) and were tested on their memory for the content of the scene, including targets that were related (e.g.,

toilet) and unrelated (e.g., vase) to its theme. Analyses focused on both similarities and differences in neural recruitment supporting memory for items related and unrelated to the schema. Correct responses to both thematically-related items and to items that were not related to the schema (non-thematic) were associated with increases in neural activity in the typical retrieval success network. A direct comparison between retrieval of thematic vs. non-thematic items found greater activity in bilateral visual and occipito-temporal regions. This was interpreted as recapitulation of items in the scene along with their surrounding thematic contexts. Additionally, results revealed greater activation in prefrontal regions associated with decision-making, evaluation and attention, as well as in middle and superior temporal gyrus when comparing non-thematic retrieval with thematic retrieval. This was interpreted as non-thematic items evoking a greater need for evaluation and decision making as participants sorted through schemas to retrieve non-thematic targets. Finally, the lack of medial temporal lobe differences between the two types of retrieval indicates that the MTL is working equally in both conditions to support retrieval success for both thematic and non-thematic information.

Disclosures: C.E. Johnson: None. **I.C. Turney:** None. **N.A. Dennis:** None.

Nanosymposium

586. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: 140A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 586.04

Topic: F.01. Human Cognition and Behavior

Support: NIMH Intramural Research Program

Title: Object information in hippocampus and visual cortex during perception and retrieval from long-term memory

Authors: *S.-H. LEE[1], M. L. KING[1], D. J. KRAVITZ[2], C. I. BAKER[1];
[1]NIH/NIMH, Bethesda, MD; [2]The George Washington Univ., Washington, DC

Abstract: Long-term memory processes allow humans to store newly learned information and recall that information later. Although it is known hippocampus critically contributes to the retrieval of long-term memories, it remains unclear how the recalled memory trace itself is represented in the hippocampus compared to cortical areas. Further, the possible role of hippocampus remains hotly debated. To compare object-specific information in hippocampus and visual cortex during perception and recall from long-term memory, we performed an event-

related functional magnetic resonance imaging (fMRI) experiment, comprising separate perception, learning and recall sessions. During the perception session, participants were scanned and presented with fixed pairings of 12 auditory cues (pseudowords, e.g. 'tenire') and object images (e.g. chair) inside the scanner. During the learning session, which took place on a separate day outside the scanner, participants were trained to memorize the pseudoword-object associations for about one hour. Finally, the following day participants were scanned and instructed to recall as vividly as possible each object image in response to the paired pseudoword cue in the absence of any visual stimulation. To test the quality of the recalled visual information, participants were asked to draw detailed pictures of the object images and perform forced-choice tests at the end of the retrieval scan session. Every participant showed good performance in the drawing and forced-choice (> 90% correct) tests indicating accurate retrieval of object information. Using multi-voxel pattern analysis, we found that during retrieval the response of both hippocampus and anterior object-selective cortex could be used to decode the identity of individual remembered objects. However, in anterior object-selective cortex but not hippocampus i) object identity could also be decoded during the perception session, ii) there was close correspondence between the representations during perception and retrieval, and iii) the accuracy and fidelity of recall (drawing test) was correlated with the decoding accuracy. These results show that recall of object information from long-term memory activates a fine-grained representation in both hippocampal and cortical areas. However, the quality of the recall is reflected only in cortex, suggesting that while hippocampus may be involved in the initiation of recall, object-selective cortex represents the fine detail of the information.

Disclosures: **S. Lee:** None. **M.L. King:** None. **D.J. Kravitz:** None. **C.I. Baker:** None.

Nanosymposium

586. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: 140A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 586.05

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant EY022350

NSF Grant SBE-0541957

Title: Anchoring the neural compass: Coding of local spatial reference frames in human medial parietal cortex

Authors: *S. A. MARCHETTE, L. K. VASS, J. RYAN, R. A. EPSTEIN;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: The neural systems that code for location and facing direction during spatial navigation have been extensively investigated, but the mechanisms by which these quantities are referenced to external features of the world are not well understood. To address this issue, we examined behavioral priming and fMRI activity patterns while human subjects re-instantiated spatial views from a newly-learned virtual environment consisting of four buildings aligned at different angles relative to each other within a larger park. The layout of this environment allowed us to dissociate local spatial representations that were aligned to each building from global spatial representations that applied across the entire space. Behavioral results indicated that imagined location and imagined heading were represented during the spatial imagery task, and multi-voxel pattern analyses indicated the retrosplenial complex (RSC) in medial parietal cortex was the anatomical locus of these spatial codes. Indeed, we were able to recreate accurate maps of the spatial relationships between imagined views based on activity patterns in RSC. Notably, location and heading codes in RSC were locally-referenced: they were aligned to the interior axis of each building rather than to the larger park. Moreover, they generalized across the geometrically-identical building interiors. These findings suggest that RSC may code location and heading relative to structural features of the local environment (such as walls). These locally-referenced spatial representations might serve to anchor our sense of direction to the fixed elements of the external world.

Disclosures: S.A. Marchette: None. **L.K. Vass:** None. **J. Ryan:** None. **R.A. Epstein:** None.

Nanosymposium

586. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: 140A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 586.06

Topic: F.01. Human Cognition and Behavior

Support: CNRS

LABEX Cortex NR-11-LABX-0042

Region Rhône-Alpes (2010)

Roudnitska Fundation

Title: Odor-evoked episodic memory in humans: From cognitive process to neural network

Authors: *A.-L. SAIVE, N. RAVEL, M. THÉVENET, S. GARCIA, J.-P. ROYET, J. PLAILLY;
Lyon Neurosci. Res. Ctr., Lyon Cedex 07, France

Abstract: Human episodic memory is the memory that permits the conscious re-experience of specific personal events from the past (Tulving, 1983) and is associated with a feeling of mental time travel (Tulving, 2001). Odors are powerful cues that trigger episodic memories. However, the paucity of information available on the functioning of odor episodic memory is startling. In this ensemble of studies, we developed a novel behavioral approach to investigate odor-evoked episodic memories being both highly controlled and as ecological as possible (Saive et al., 2013). The participants freely explored three unique and rich laboratory episodes, one episode per day; each episode consisted of three unfamiliar odors (What) positioned at three specific locations (Where) within a visual context (Which context). During the retrieval test occurring the next day, odors were used to trigger the retrieval of the complex episodes (participants were either at the laboratory or within the fMRI scanner). The participants were proficient in recognizing the target odors among distractors and retrieving the visuospatial context in which they were encountered. The episodic nature of the task generated high and stable memory performances, influenced by the emotional content of the odors, with both pleasant and unpleasant odors generating higher recognition and episodic retrieval than neutral odors. The findings also suggested that when the binding between the odors and the spatio-contextual features of the episode was successful, the odor recognition and the episodic retrieval collapsed into a unique memory process that began as soon as the participants smelled the odors (Saive et al., in revision). The fMRI approach enabled the investigation of the neural signature of the successful retrieval of episodic memories from the odor perception to the episodic elaboration. Preliminary findings suggest the early involvement of episodic retrieval network from the mere odor perception. Saive, A.-L., Ravel, N., Thévenet, M., Royet, J.-P., and Plailly, J. (2013). A novel experimental approach to episodic memory in humans based on the privileged access of odors to memories. J. Neurosci. Meth. 213, 22-31. Saive, A.-L., Royet, J.-P., Ravel, N., Thévenet, M., Garcia, S., and Plailly, J. A unique memory process modulated by emotion underpins successful odor recognition and episodic retrieval in humans. in revision. Tulving, E. (1983). Elements of episodic memory. Oxford: Clarendon. Tulving, E. (2001). Episodic memory and common sense: how far apart? Philos. Trans. R. Soc. Lond., B, Biol. Sci. 356, 1505-1515.

Disclosures: **A. Saive:** None. **N. Ravel:** None. **M. Thévenet:** None. **S. Garcia:** None. **J. Royet:** None. **J. Plailly:** None.

Nanosymposium

586. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: 140A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 586.07

Topic: F.01. Human Cognition and Behavior

Support: ERC-2012-ADG, SH4. FP7-IDEAS (M4)

ANR-12-BSH2-0010 (ELMA)

Title: Echoic memory for meaningless stimuli: Evidence for fast neuronal specialization?

Authors: *J. VISWANATHAN[1,2], N. MACÉ[1,2], A. S. CAUQUIL[1,2], S. J. THORPE[1,2], F. REMY[1,2];
[1]CerCo, CNRS UMR 5549, Toulouse, France; [2]Univ. Toulouse III - Paul Sabatier, Toulouse, France

Abstract: *Introduction:* As recently shown, we are remarkably good at recognizing patterns in auditory Gaussian noise samples following implicit encoding of these meaningless stimuli (Agus, Thorpe, & Pressnitzer, 2010). We aimed to test the robustness of this long-term memory trace to acoustic transformation of noise samples. Moreover, the neural basis of memory for noises was explored using a combined EEG and fMRI experiment. *Methods:* The frozen noise paradigm (Guttman, N., and Julesz, 1963) allows subjects to extract specific features from random noise, and these features cannot be rehearsed. We used an implicit learning task, where subjects (n=20) had to differentiate noises containing repeated segments vs. plain random noises. The learning phase included 10 blocks, with some noise samples recurring 20 times throughout a block (reference noises). Subjects were tested again 4 weeks later to measure retention of the 10 reference noises, when intact or transformed (scrambling of short segments or temporal jitter). In some subjects, long-term retention of implicitly learnt reference noises was tested in a combined fMRI-EEG set-up, in order to identify the temporal and spatial bases of memory traces for meaningless stimuli. *Results:* In the retention test, subjects' accuracy was high for some of the reference noises right from the first trial, showing that these noises were learnt. Moreover, performance remained high when these noises were temporally modified, and depended on the quality of learning. Analysis of fMRI data in pilot subjects evidenced higher activity in the thalamus, primary auditory cortex and in parietal regions for unlearnt vs. learnt reference noises. *Conclusions:* A long-term memory trace for some specific noise features, robust to acoustic transformation, was demonstrated. During retention, unlearnt noises might elicit greater attention than learnt noises, although previous exposure to these noises was strictly equivalent. Moreover, decreased activity in the thalamus and auditory cortex in response to learnt noises might reflect neuronal specialization to specific acoustic features.

Disclosures: **J. Viswanathan:** None. **N. Macé:** None. **A.S. Cauquil:** None. **S.J. Thorpe:** None. **F. Remy:** None.

Nanosymposium

586. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: 140A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 586.08

Topic: F.01. Human Cognition and Behavior

Support: Medical Research Service of the Department of Veterans Affairs

NIMH MH24600

NSF SMA-1041755

Title: Hippocampal brain activity and functional connectivity in humans change as material is forgotten across one month

Authors: ***C. N. SMITH**[1,4], R. E. CLARK[1,2,4], L. R. SQUIRE[1,2,3,4];
[1]Psychiatry, [2]Neurosciences, [3]Psychology, UCSD, San Diego, CA; [4]Veterans Affairs San Diego Healthcare Syst., San Diego, CA

Abstract: Memories associated with notable news events are strong and are forgotten only gradually over a lifetime. Findings from patients with hippocampal damage indicate that memory for news events becomes hippocampus-independent within a few years after learning. Correspondingly, fMRI activity in the healthy hippocampus sharply decreases over this same time course. These findings are consistent with the predictions of systems consolidation whereby the brain systems that support memory retrieval change as a function of memory age. We examined brain activity and brain functional connectivity in humans in relation to memory age for material forgotten relatively quickly (over the course of one month). Fifteen participants studied photos of indoor and outdoor scenes. Each participant studied four sets of 80 photos, one on each of 4 occasions prior to scanning (1 hour, 1 day, 1 week, and 1 month). Memory for the scenes was then tested during a single fMRI session where participants saw old and new scenes and made old/new recognition judgments and gave confidence ratings. Memory and confidence decreased sharply over the course of one month. Unexpectedly, hippocampal activity increased as time passed after learning. This finding may be related to re-encoding of the more poorly remembered remote items. We also found that hippocampal functional connectivity with

neocortex and parahippocampal gyrus decreased sharply over time, following the forgetting rate. In summary, hippocampal activity and connectivity changed sharply across time according to the forgetting rate. Measures of functional connectivity may better reflect how the brain stores memory than measures of activity, which may more susceptible to factors other than memory retrieval (e.g., re-encoding of poorly remembered study material).

Disclosures: **C.N. Smith:** None. **R.E. Clark:** None. **L.R. Squire:** None.

Nanosymposium

586. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: 140A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 586.09

Topic: F.01. Human Cognition and Behavior

Support: BMBF 01GQ1003B

Title: Relative and absolute familiarity are associated with two topographically dissociable early ERP old/new effects

Authors: ***R. BADER**, P. MEYER;
Central Inst. for Mental Hlth., Mannheim, Germany

Abstract: Dual process-models of recognition memory assume that two distinct cognitive processes underlie the ability to judge the prior occurrence of an object, person, or event: recollection and familiarity. Whereas recollection is defined as the memory for specific episodic details, familiarity is described as a feeling of knowing without memory for episodic details. Familiarity has been associated with the event-related potentials (ERP) early mid-frontal old/new effect. Occurring 300 to 500 ms after stimulus onset and being most pronounced over frontal sites of the scalp, the mid-frontal old/new effect has topographically and temporally been dissociated from the later occurring recollection-related left parietal old/new effect. However, its exact functional significance is unclear to date. Some studies proposed that it reflects the relative familiarity after a study event, i.e. the increase in familiarity strength relative to a pre-study baseline. When word pairs are studied for the first time together with a definition forming a novel conceptual unit (e.g., the word pair MILK TAXI is studied together with the definition “A delivery service which is directly dispatched from a farm”), the assessment of absolute familiarity is sufficient. In contrast to relative familiarity, absolute familiarity considers if an item has ever been occurred before, no matter when. In this case, the early old/new effect has

been found to exhibit a more posterior distribution than the mid-frontal old/new effect. To test whether the transition from an absolute to a relative familiarity signal in response to identical stimulus material is associated with a shift in topography of the early ERP old/new effect, we compared old/new effects for novel conceptual units at two different stages of learning. At day 1, when they have just been learned, familiarity-based recognition decisions must rely on absolute familiarity. After this first encounter, the novel concepts were rehearsed additional three times. One week later (day 2), participants studied a part of the rehearsed pairs again and during the test phase, they had to discriminate them from the non-studied portion of the rehearsed pairs. At day 2, recognition memory can only be subserved by relative familiarity as absolute familiarity is no longer diagnostic. We found that memory accuracy is higher and reaction times are faster on day 2 than on day 1. Moreover, the early old/new effect on day 2 has a more anterior topographical distribution than the effect on day 1, which has a posterior maximum. This is consistent with the notion that novel conceptual units and pre-existing items are associated with two different kinds of familiarity signals.

Disclosures: R. Bader: None. **P. Meyer:** None.

Nanosymposium

586. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: 140A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 586.10

Topic: F.01. Human Cognition and Behavior

Title: Memory representation strength modulates the neural networks supporting associative recognition and novelty detection

Authors: *Y. FANDAKOVA[1], M. C. SANDER[2], R. CABEZA[3], U. LINDENBERGER[2], M. WERKLE-BERGNER[2], Y. L. SHING[2];
[1]Univ. of California, Davis, CA; [2]Max Planck Inst. for Human Develop., Berlin, Germany; [3]Duke Univ., Durham, NC

Abstract: Remembering past episodes activates a broad network of medial temporal (MTL), prefrontal (PFC), and parietal regions. To date, little is known about the extent to which the strength or quality of memory representations modulates the involvement of these networks during retrieval. In the present study, 27 participants aged 20-27 years studied 440 unrelated scene-word pairs. Following initial presentation of the pairs, each picture served as a cue and participants had to recall the associated word. Independent of recall accuracy, the correct word

was presented again, presumably eliciting further associative learning. Participants then completed a criterion cued-recall task without feedback. Each individual's performance on the criterion cued-recall task (M = .55, SD = .13) was used to distribute the scene-word pairs for an associative recognition test that took place approximately 24 hours later. Specifically, participants underwent fMRI scanning during associative recognition of exact repeats of previously studied scene-word pairs along with rearranged and novel pairs. Half of the exact repeats and rearranged pairs were drawn from pairs for which participants had correctly recalled the corresponding word when cued with the picture in the criterion cued-recall task on the previous day (i.e., strong representations), whereas the other half were drawn from pairs for which participants had not correctly recalled the corresponding word (i.e., weak representations). Strong representations were associated with better recognition performance on both exact repeats (strong = .96, weak = .64) and rearranged pairs (strong = .95, weak = .77). Preliminary whole-brain analyses revealed that correct detection of weak relative to strong exact repeats activated a fronto-parietal network including right dorsal anterior cingulate cortex, left dorsolateral and anterior PFC, as well as left superior parietal lobe. In contrast, correct detection of strong relative to weak exact repeats engaged bilateral ventromedial PFC, temporo-parietal junction and precuneus. These results suggest that mnemonic control processes are selectively engaged in the retrieval of weak representations, whereas stronger memories are accompanied by a less pronounced deactivation of the default mode network. Relative to the correct rejection of rearranged pairs based on weak representations, the correct rejection of rearranged pairs based on strong representations was associated with increased bilateral hippocampal activation. This finding suggests that associative novelty signals in this region are modulated by the strength of the representations that are combined in a novel way.

Disclosures: Y. Fandakova: None. **M.C. Sander:** None. **R. Cabeza:** None. **U. Lindenberger:** None. **M. Werkle-Bergner:** None. **Y.L. Shing:** None.

Nanosymposium

586. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: 140A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 586.11

Topic: F.01. Human Cognition and Behavior

Support: EU FP7 (Grant agreement 604102)

Israeli Science Foundation

Foundation Adelis

Human Frontier Science Program long-term fellowship

Title: Word length effect in free recall of randomly assembled word lists

Authors: *M. KATKOV[1], S. ROMANI[2], M. TSODYKS[1];
[1]Neurobio. department, Weizmann Inst. of Sci., Rehovot, Israel; [2]Ctr. for Theoretical Neurosci., Columbia Univercity, New York, NY

Abstract: In serial recall experiments, human participants are requested to retrieve a list of words in the same order as they were presented. In a classical study, participants were reported to recall more words from study lists composed of short words compared to lists of long words, the word length effect. Word length effect is considered to be one of the key phenomena in the theories of short-term memory, and is traditionally explained by limited capacity of short-term memory combined with either increased complexity or increased rehearsal time of longer items. It is, therefore, assumed that fewer long words can be stored in short-term memory, leading to reduced performance. The world length effect was also observed in long-term memory tasks, where lists are repeatedly presented to participants until all words are retrieved in correct order, and in free recall experiments, where participants can retrieve the words in any order. Those observations suggest that more general mechanism, not explicitly employing short-term memory, is involved in word length effect. Recently we have proposed a mechanism of associative information retrieval that explicitly takes into account long-term neuronal representations of memory items, that does not involve separate short-term memory mechanism. One of the basic predictions of the model is the existence of "easy" and "difficult" words. This prediction was verified in our analysis of large dataset of free recall experiments collected in the lab of Michael Kahana, where we showed that the probability of words to be recalled are consistent between arbitrarily chosen groups of participants. Here we studied what if any is the contribution of the word length to the intrinsic difficulty of the word to be recalled. Toward this end, we further analyzed the dataset, where short and long words were randomly mixed, a paradigm that was not used to study word length effect before. We found a seemingly opposite effect: long words were recalled better than the short ones (correlation coefficient between number of syllables in the word and frequency of the same word being recalled when presented is 0.15, $p< 10^{-6}$). Moreover, for groups of words with the given number of syllables, both mean and variance of recall probabilities were monotonic function of number of syllables. Finally, we found that our recently proposed mechanism of associative retrieval can explain both groups of effects. The additional assumption used in the model is an increased variance in the number of encoding neurons with increased length of the words. Furthermore, the direction of the word length effect depends solely on the way study lists are composed.

Disclosures: **M. Katkov:** None. **S. Romani:** None. **M. Tsodyks:** None.

Nanosymposium

586. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: 140A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 586.12

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant RO1 MH060941

Title: How shifts in visual perspective modify autobiographical memory: A functional neuroimaging study

Authors: *P. L. ST. JACQUES;
Psychology, Harvard Univ., Cambridge, MA

Abstract: Autobiographical memories (AM) can be retrieved from a first person perspective (i.e., through one's own eyes) or from a third person perspective (i.e., as an outside observer), which affects the phenomenological experience and content of memories during retrieval. It is not well understood whether differences in visual perspective during retrieval lead to long-term changes in memory, and the neural mechanisms that support these potential changes. In the current fMRI study we used a repetition suppression design in order to identify the neural mechanisms by which shifts in visual perspective from a first to a third person perspective during retrieval can modify long-term memory. Participants generated AM approximately one week prior to fMRI scanning, and we selected AM associated with a strong first person perspective (i.e., based on subjective ratings of >= 5 on a 7-point scale of the amount of first person perspective AND <4 on a 7-point scale of the amount of third person perspective). During scanning, participants retrieved a subset of these strong first person memories from the same perspective (i.e., first person) or from an different perspective (i.e., third person), three times for each memory in each functional run. Two days later, participants retrieved all of the AM from a naturally occurring perspective, including memories that were retrieved or not retrieved (i.e., baseline) during scanning, and to report the visual perspective. The preliminary behavioral results revealed that memories repeatedly retrieved from a different perspective compared to the same perspective were associated with greater changes in self-reported visual perspective from the initial session to the final session. The fMRI results revealed robust repetition suppression effects that were similar in the same and different perspective conditions in a number of regions within the default network, including the lateral parietal and retrosplenial cortices, and the medial temporal lobe (hippocampus and parahippocampal cortices). In sum, our behavioral results suggest that shifts in visual perspective can lead to long-term modification of AM, and we

will discuss how such modification is supported by similar and different neural mechanisms during perspective shifting.

Disclosures: P.L. St. Jacques: None.

Nanosymposium

586. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: 140A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 586.13

Topic: F.01. Human Cognition and Behavior

Title: Remembering exceptions to the rule: Dorsolateral PFC and Striatum support memory for schema-incongruent events

Authors: *G. BROD, U. LINDENBERGER, M. WERKLE-BERGNER, Y. L. SHING;
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Abstract: Introduction Recent studies in rodents and humans have shown that the medial prefrontal cortex (mPFC) is involved in encoding new information that is congruent to a pre-existing schema and supports its retrieval. The present study examined differences between remembering information that is congruent to an existing schema and information that is related but incongruent to a schema. As differences between congruent and incongruent events may vary across people for tasks utilizing world knowledge, we used a novel paradigm that experimentally induces knowledge in the form of a hierarchy. Methods Data of 21 young adults were analyzed. The experiment consisted of two sessions of about 2 hours each, taking place on two consecutive days. On day 1, participants learned a hierarchy of novel objects. On day 2, this knowledge then served as the schema in a memory task that involved remembering outcomes that were either congruent or incongruent with the hierarchy. For the fMRI analysis, we focused on differences in brain activity and connectivity during retrieval as modulated by congruency. To get at the interaction between congruency and memory, we examined brain regions where differences between remembered and forgotten trials (i.e. memory effect) were larger in the congruent than in the incongruent condition, and vice versa. Results The interaction analysis revealed stronger mPFC activity for congruent remembered items and stronger dorsolateral PFC (DLPFC), parietal, and striatal activity for incongruent remembered items. Based on these findings, psychophysiological interaction (PPI) analyses were performed with seeds in the HC and the striatum. Striatum showed significant co-activation with DLPFC, which was stronger for remembered incongruent outcomes than remembered congruent outcomes. In addition, this

increase in connectivity was stronger in participants with better memory for incongruent events. No significant effects were observed for the hippocampal seed. Conclusions We extend previous findings by showing that the mPFC plays a key role in retrieving information that is congruent with an experimentally generated schema. Memory for incongruent events, on the other hand, is related to a network involving the DLPFC and the striatum. We argue that remembering schema-related incongruent events requires remembering the specific situational context of the encoding situation and overcoming biases from the schema, which is reflected in enhanced activities in the DLPFC, parietal cortex, and striatum, and enhanced connectivity between striatum and DLPFC.

Disclosures: G. Brod: None. **U. Lindenberger:** None. **Y.L. Shing:** None. **M. Werkle-Bergner:** None.

Nanosymposium

586. Human Long-Term Memory: Encoding-Retrieval Interactions

Location: 140A

Time: Tuesday, November 18, 2014, 1:00 PM - 4:30 PM

Presentation Number: 586.14

Topic: F.01. Human Cognition and Behavior

Title: Episodic memory retrieval benefits from a less modular brain network organization

Authors: *A. J. WESTPHAL, M. M. MONTI, N. REGGENTE, O. YAZDANSHENAS, J. RISSMAN;
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Abstract: The act of retrieving a memory for a specific episode of one's past requires the coordination of brain networks involved in controlling access to mnemonic contents and representing and monitoring the stored information. This has been shown to invoke a brain connectivity profile that diverges somewhat from the brain's intrinsic resting state organization (Fornito et al., 2012). However, it is not yet clear to what degree this "retrieval mode" brain state differs from that observed during other complex cognitive tasks. In order to examine this further, we performed a graph theoretical analysis on fMRI functional connectivity data patterns measured while participants (N = 20) alternated between the performance of episodic source memory retrieval, analogical reasoning, and visuospatial perception tasks. In order to avoid systematic confounds, we ensured that the tasks were matched for response demands, reaction times, and bottom-up visual processing. Following preprocessing, we extracted fMRI time-courses from each 40 sec task block and concatenated these across runs to generate task-specific time-courses. We next reduced our whole brain data set to 264 functional areas, identified by

resting state parcellation and meta-analysis (Power et al., 2011) and defined as spherical regions of interest (5mm radius). Pairwise correlations were then computed between all pairs of nodes for each cognitive task, and the weakest connections were thresholded out at a range of sparsity values. To capture a key global property of brain network dynamics, we analyzed how much each task-set expressed a graph theoretic measure known as modularity (Newman, 2006), which assesses the amount of connectivity within identified networks versus between networks. Our data revealed that the memory retrieval task showed significantly reduced modularity in comparison to the reasoning and perception tasks, an effect that replicated across sparsity thresholds. This suggests that the memory task-set is characterized by more widespread connectivity across the brain. Strikingly, reduced modularity in individual subjects was diagnostic of fewer memory errors and improved source monitoring. Taken together, our results suggest that memory retrieval may benefit from lower modularity, presumably because otherwise competitive brain networks supporting externally-directed and internally-directed attention must work together to link environmental stimuli with an introspective mnemonic search process.

Disclosures: A.J. Westphal: None. **M.M. Monti:** None. **N. Reggente:** None. **O. Yazdanshenas:** None. **J. Rissman:** None.

Nanosymposium

587. Human Decision-Making: Neural Mechanisms

Location: 150A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 587.01

Topic: F.01. Human Cognition and Behavior

Title: Rapid intermittent deep brain stimulation biases behavior in financial decision-making task

Authors: *S. R. PATEL[1], S. SHETH[2], M. MIAN[2], S. BOURNE[2], J. YANG[2], E. ESKANDAR[2]; [1]Dept. of Neurosurg., [2]Massachusetts Gen. Hosp., Boston, MA

Abstract: We report single-unit responses recorded from the human subthalamic nucleus (STN) in patients undergoing deep brain stimulation while engaged in a financial decision-making task. The task is modeled as a simplified version of the classic card game, “war”. The subject is dealt a card and asked to make a high or low wager ($5 or $20). Immediately following their choice they are shown their opponent’s card--the player with the highest card wins. We recorded 20 individual neurons from 5 patients. We found that during the go-cue period, neuronal activity in a 500 ms window predicted whether subjects would ultimately bet high or low on trials where

the probability of a positive or negative outcome was equal (6-card trials, two-tailed t-test, p = 0.03). To explore this further, in a second experiment, we applied intermittent electrical stimulation within this window to selectively modulate decision-making. From 15 subjects, using a modified stimulator, we applied one of three stimulation conditions during 6-card trials: no stimulation, 1 sec of stimulation at the fixation, or 1 sec of stimulation at the go-cue epoch. We found that intermittent stimulation at the go-cue epoch--the same period STN neurons encode the upcoming decision--biased subjects to make a low wager (binomial proportion, 95% c.i.). Fixation and no stimulation categories had no effect on decision-making. In this study, we demonstrated that neuronal activity in the dorsal STN predicts financial decisions. We then showed that we could apply intermittent electrical stimulation through the implanted deep brain stimulation electrode to bias the decision signal and ultimately alter subject behavior.

Disclosures: S.R. Patel: None. **S. Sheth:** None. **M. Mian:** None. **S. Bourne:** None. **J. Yang:** None. **E. Eskandar:** None.

Nanosymposium

587. Human Decision-Making: Neural Mechanisms

Location: 150A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 587.02

Topic: F.01. Human Cognition and Behavior

Title: Serotonin transporter gene (5-HTTLPR) modulates the neural activation of ventromedial prefrontal cortex in loss aversion

Authors: *Q. HE[1], G. XUE[2], C. CHEN[2], Q. DONG[2], A. BECHARA[3];
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Abstract: Individuals vary greatly in on the level of loss aversion and such may have genetic bases. We reported that 5-HTTLPR polymorphism influenced loss aversion in a large sample (He et al., 2010). In this study, we recruited an independent sample to test the underlying neural mechanism. Fifty-eight (32 female, 14 l/l, 24 l/s, and 20 s/s) healthy Chinese college students (aged 20.1 ± 1.2 years) were recruited. The task design has been described in detail elsewhere (Tom et al., 2007; He et al., 2010). Briefly, participants were presented with 3 runs (8 minutes each) of 85-86 trials, each of which proposed a mixed gamble entailing a 50/50 chance of gaining one amount or losing another amount of real money. Consistent with our previous result, subjects showed indifference to gambles in which potential gains were twice as likely as losses,

and they also needed more time to decide whether or not to accept those gambles. Conjunction analysis between increasing activity for gains and decreasing activity for losses demonstrated joint sensitivity to both gains and losses in a set of brain regions, including left postcentral gyrus, left posterior insula, ventromedial prefrontal cortex (VMPFC), right cerebellum, and subcallosal cortex. Also, several brain regions showed the pattern of neural loss aversion, including VMPFC (extending to bilateral striatum, left amygdala and left insula), left postcentral cortex, posterior cingulate cortex (PCC), precuneous, and cerebellum. These results replicate results of Tom et al. (2007) in a larger sample. To identify regions whose activity was modulated by 5-HTTLPR genotype, we tested the genotype difference on three main contrasts: sensitive to gains, sensitive to losses, and neural loss aversion. Results suggested the VMPFC region showed genotype difference in all three conditions. There's great overlap between sensitive to gains and neural loss aversion as well as between sensitive to losses and neural loss aversion, but there is no overlap between sensitive to gains and losses. ROI analysis showed that the activation in s/s group was higher (lower for sensitive to losses) than l/l group with l/s falls in between. The present study replicate the behavior (He et al., 2010) and fMRI finding (Tom et al., 2007) in previous studies. We found that 5-HTTLPR genotype modulates the neural activity of the VMPFC region in loss aversion: the activation in s/s group was higher (lower for sensitive to losses) than l/l group with l/s falls in between. These results suggested that the neural response of VMPFC could serve as an endophenotype connecting gene (5-HTTLPR polymorphism) and behavior (loss aversion) in decision making.

Disclosures: **Q. He:** None. **G. Xue:** None. **C. Chen:** None. **Q. Dong:** None. **A. Bechara:** None.

Nanosymposium

587. Human Decision-Making: Neural Mechanisms

Location: 150A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 587.03

Topic: F.01. Human Cognition and Behavior

Support: Fundación La Caixa

Title: Neural correlates of effort-discounted value and effort-based uncertainty in human decision making

Authors: *J. BERNACER[1], I. MARTINEZ-VALBUENA[1], M. MARTINEZ[2], N. PUJOL[2], M. A. PASTOR[2];
[1]Mind-Brain Group, [2]Univ. De Navarra, Pamplona, Spain

Abstract: When making a decision, humans choose the option with the highest value. However, this value is far from being objective; rather, it is devalued by different factors, namely risk (odds to obtaining the reward), time (delay on getting it) and effort (the physical energy expenditure to obtain it). Whereas risk and time have been extensively studied in neuroscience, the impact of a prospective effort on decision making remains relatively unexplored. Thus, our study aims to clarify: 1) the behavioral underpinnings of effort as a discounting factor; 2) the neural correlates of subjective value and uncertainty (measured as Shannon entropy), when a reward is devalued by a prospective effort. To achieve this goal, we recruited 40 volunteers that performed a decision-making task involving different amounts of money and different levels of a prospective effort (minutes running in a treadmill) to earn it. In addition, a sub-sample of 17 subjects was included in an fMRI experiment to assess the neural correlates of subjective value and uncertainty in a decision-making task that involved effort as principal devaluator: while being scanned, subjects were presented pairs of options that differed in probability (odds to win) and effort (minutes running in a treadmill) to earn a fixed payment (30 euros). The first part of the experiment revealed that, considering the whole sample, a prospective effort discounted the objective value of money closely following a hyperbolic model (R_2 adjusted=0.9989). However, the individual devaluation of most of the subjects (n=20) was exponential (hyperbolic, n=17; double exponential, n=4). Our neurocomputational fMRI voxel-based analysis revealed that subjective value across trials was correlated with BOLD signal in the ventrolateral prefrontal cortex, posterior caudate nucleus and putamen, posterior insula and precentral gyrus of the right hemisphere (Family Wise Error, cluster-wise corrected at $z<2.3$, $P=0.05$). Furthermore, effort-based uncertainty quantified as Shannon entropy was coded in the anterior paracingulate/supplementary motor area (same statistical threshold). In all, our study demonstrates that physical effort follows a discount function alike to risk and time, and its brain correlates are partly shared and partly distinctive with respect to other devaluators. Supported by Fundación La Caixa.

Disclosures: J. Bernacer: None. **I. Martinez-Valbuena:** None. **M. Martinez:** None. **N. Pujol:** None. **M.A. Pastor:** None.

Nanosymposium

587. Human Decision-Making: Neural Mechanisms

Location: 150A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 587.04

Topic: F.01. Human Cognition and Behavior

Support: NSF CAREER SES 1352632

NSF CMMI 1200830

NSF SES 1230933

Title: Using metabolic cost to determine the subjective value of effort in movement decisions

Authors: *E. SUMMERSIDE, A. AHMED;
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Abstract: Nearly every movement we make involves a decision. Similar to an economic decision, a movement decision seeks to maximize rewards and minimize costs. However, instead of monetary costs, the predominant cost in movement is effort. In economic decision-making, it has long been realized that it is not solely the objective monetary cost that influences the decision, but the subjective value that an individual associates with that cost. Surprisingly, we do not yet have a good representation of the subjective value the brain assigns effort. Here, we seek to address this gap in our knowledge and quantify the subjective value the brain assigns effort. Specifically, we measured objective effort (i.e. metabolic) cost directly, via expired gas analysis. We then tested the hypothesis that there will be a distortion between subjective effort valuation and objective effort cost. Participants (n=13) participated in two separate sessions. The first session involved performing 20cm reaches, during which metabolic rate was measured via expired gas analysis. Participants reached against five different resistances. In the second session, they made choices between a sure bet of performing 5min of low effort reaches or risk performing higher effort reaches. The risky choice was presented as a percentage value paired with one of the five resistances. The percentage represented the chance of having to reach for 5min at the presented resistance with the alternative outcome being sitting quietly for 5min. Using the metabolic data and the individual's choice data, the subjective valuation of effort was fitted using Cumulative Prospect Theory. Effort cost was fitted to α and signified if a subject tended to over value ($\alpha > 1$), undervalue ($\alpha < 1$) or accurately value ($\alpha = 1$) the effort required to complete the arm-reaching task. We were able to measure differences in the metabolic cost of arm reaching under increasing resistances in all subjects. Considering metabolic rate as the effort cost, subjects tended to have a subjective valuation of effort that was different than the objective cost. The average α across subjects was 1.29 +/- 0.28 (95CI). Of the 13 subjects, the majority (10) overvalued the cost of effort ($\alpha > 1$). It appears that in an arm reaching task, individuals tend to overvalue the cost of effort. The observed distortion and variability among participants should be taken into consideration in models of movement decision making to allow for more accurate predictions and improved understanding of the underlying process. Further development of this novel approach may also lead to a better understanding of how best to communicate effort costs to improve decision making.

Disclosures: E. Summerside: None. **A. Ahmed:** None.

Nanosymposium

587. Human Decision-Making: Neural Mechanisms

Location: 150A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 587.05

Topic: F.01. Human Cognition and Behavior

Support: Wellcome Trust

Title: Individual differences in belief formation predicted by frontal-subcortical connectivity

Authors: *C. MOUTSIANA[1], N. GARRETT[1], C. CHARPENTIER[2], M. X. COHEN[3], T. SHAROT[1];
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Abstract: We are constantly swamped with information that forms our beliefs about reality (e.g., via news outlets, social media, family and peers). However, even when presented with accurate information, cognitive biases and heuristics can limit our ability to make suitable adjustments to our prior beliefs. Understanding the biological factors that underlie individual differences in this domain is critical, not only because biased belief formation is a hallmark of mental diseases (e.g., depression, schizophrenia), but also because individuals' inaccurate beliefs considerably impact society (e.g., effecting financial markets and politics). The most salient attribute of information is valence; whether information is favourable or unfavourable is vital in predicting if it will alter beliefs. Here, we reveal a striking valence-dependent asymmetry in how frontal-subcortical connectivity is related to belief formation. Using diffusion tensor imaging (DTI) we show that individuals with stronger anatomical connections linking the left Inferior Frontal Gyrus (IFG) with a network of subcortical regions (including the striatum, amygdala, hippocampus and thalamus) are more likely to alter their beliefs in response to good news but less likely to alter their beliefs in response to bad news. The effect is specific to the left hemisphere. Our findings provide the first evidence that frontal-subcortical connectivity predicts belief formation in opposite directions depending on the valence of the new information. The results are consistent with models suggesting that different computational and neural processes govern learning from good and bad news, and suggest that the strength of left frontal-limbic connectivity underlie the effect of motivation on belief.

Disclosures: C. Moutsiana: None. **N. Garrett:** None. **C. Charpentier:** None. **M.X. Cohen:** None. **T. Sharot:** None.

Nanosymposium

587. Human Decision-Making: Neural Mechanisms

Location: 150A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 587.06

Topic: F.01. Human Cognition and Behavior

Support: ERC-2009-AdG#250106 to E. K.

Title: Integration of values and information in decision-making

Authors: *M. ROUAULT[1], J. DRUGOWITSCH[1,2], E. KOECHLIN[1];
[1]INSERM - Ecole Normale Supérieure, Paris, France; [2]Dept. des Neurosciences Fondamentales, Univ. de Genève, Genève, Switzerland

Abstract: Decision-making relies on evaluating action outcomes for subsequent action. Action outcomes, however, may convey two types of value signals: - *Rewarding* values (Rv), representing the valorisation of action outcomes over a continuum of subjective preferences, such as sugary or monetary values - *Informational* values (Iv), modulating subjects' belief about the appropriate action in a given situation. Rv stems from reinforcement learning models (RL), whereas Iv stems from Bayesian models. Previous experimental paradigms usually confounded Rv and Iv: a higher reward informs about more appropriate choices. Here we present a probabilistic reversal learning task aiming at dissociating Rv and Iv. Prospect theory models (Kahneman & Tversky, 1979, *Econometrica*) assume that subjects maximize expected value, but explain the suboptimality of behaviour by distortions in probabilities and rewards representations. However, they provide no psychological origin for these distortions. We test the hypothesis that, instead of distortions, subjects combined Rv and Iv in a different manner, with no computation of expected values. In our task, healthy human subjects had to decide between two targets representing two underlying states, one of which was more frequently rewarded than the other one. The potential rewards to gain for each target were displayed before choice. Crucially, we manipulated the reward distributions underlying each target to dissociate Rv and Iv. In a first condition, Rv and Iv were uncorrelated, such that values provide no information about the most frequently rewarded state. In a second condition, Rv and Iv were correlated. In a third condition, Rv and Iv were anti-correlated, meaning that higher rewards were associated with the less frequently rewarded state. Logistic regressions analyses show that : 1) Subjects extracted Iv from potential rewards presented before choice, indicating that rewards presented as cues influence subjects' belief, rather than being processed as Rv and 2) Subjects' choices were influenced by both Iv and Rv, but without computing expected values (Iv x Rv). Prospect theory models fit our behavioral data better than many alternative models. However, a computational

model integrating two parallel systems: - RL, dealing with Rv - Bayesian inference, dealing with Iv fits behavioral performance even better. This result suggests that distortions might be better explained by an integration of two systems for decision-making. Preliminary functional magnetic resonance imaging results show cerebral activations consistent with this computational model integrating concurrent RL and Bayesian inference.

Disclosures: **M. Rouault:** None. **E. Koechlin:** None. **J. Drugowitsch:** None.

Nanosymposium

587. Human Decision-Making: Neural Mechanisms

Location: 150A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 587.07

Topic: F.01. Human Cognition and Behavior

Support: Wallenberg Foundation

Title: A predictive model of the task environment tunes control processes during contextual decision-making

Authors: ***M. L. WASKOM**, A. D. WAGNER;
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Abstract: Many decisions require a context-dependent mapping from sensory evidence to action. Although cognitive control can be used to enable this flexibility, control processes are both costly and limited. When the structure of the environment favors one decision context, it would thus be advantageous to configure the decision-making system to prioritize it. There is considerable evidence that the brain learns from its environment to support predictions about reward. We hypothesized that decision-making processes are tuned by a similar predictive model that learns over representations of task context; control is then required when events diverge from the predictions of this model. To explore this idea, we scanned human participants while they performed a context-dependent perceptual decision task. Participants were cued on each trial to judge either the direction of coherent motion or the dominant color of a noisy random dot stimulus. Within this paradigm, we parametrically manipulated the relative frequency of motion and color trials over different task epochs, although we did not indicate to participants which context was more likely at any given time. To perform optimally, participants should infer the current structure from recent experience and configure their decision-making to favor the more likely context. We formalized this learning process with a Bayesian ideal observer model, which

allowed us to define a metric of context prediction error (CPE). If control is recruited in response to violations of a predictive model, trials with larger CPEs would require more top-down influence to effectively complete. Behavioral and model-driven fMRI analyses supported this hypothesis: both reaction times and the amplitude of task-evoked activation in the frontoparietal control network parametrically scaled with CPE. To further study the effects of CPE on control mechanisms, we used a targeted dimensionality reduction of prefrontal population activity. This allowed us to define an axis corresponding to the task context representation in the population response space. The projection of population activity onto the context axis measures the influence of top-down processes on decision-making. We found that trials with high CPE were more separated along this axis, further supporting the theory that control is engaged in response to CPEs. Together, these results provide a novel computational perspective on mechanisms of context-dependent decision-making.

Disclosures: M.L. Waskom: None. **A.D. Wagner:** None.

Nanosymposium

587. Human Decision-Making: Neural Mechanisms

Location: 150A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 587.08

Topic: F.01. Human Cognition and Behavior

Support: Medical Research Council

Department of Health National Institute for Health Research Oxford Biomedical Research Centre

Title: Human subthalamic nucleus theta coherence and spike phase locking delay decision making during conflict

Authors: *B. A. ZAVALA[1], P. BROWN[2], K. ZAGHLOUL[1];
[1]Surgical Neurol. Br., Natl. Inst. of Neurolog. Dis. and Str, Bethesda, MD; [2]Clin. Neurol., Univ. of Oxford, Oxford, United Kingdom

Abstract: Recent studies have provided increasing experimental support that the basal ganglia, with their direct connections to the cortex, play an important role in decision making by adjusting the temporal dynamics of motor control. Critical to this architecture is the subthalamic nucleus (STN), which may increase activity and thereby inhibit movements during difficult

decisions. Despite experimental support for this role, however, the precise neural mechanisms by which the STN delays responses during difficult decisions are not fully understood. To investigate this, we conducted three separate experiments involving local field and single unit recordings from the human STN while participants engaged in sensorimotor decision tasks. We first captured STN local field potential (LFP) signals from externalized deep brain stimulation (DBS) leads as participants performed an Eriksen flanker task. We found significantly greater theta oscillatory power during trials that involved high sensory decision conflict compared to trials that involved low conflict. We also found that theta oscillatory power correlated with individual trial reaction time. We next recorded simultaneous frontal scalp EEG and STN LFP signals, captured from externalized DBS leads, as a second group of participants performed a sensorimotor decision task involving identifying the average direction of randomly moving dots. We found that during trials in which two populations of dots moved in conflicting directions, the STN exhibited significant increases in theta oscillatory power and significant theta band synchrony with the frontal cortex. Importantly, theta oscillations in the frontal cortex were Granger causal to those in the STN. Finally, in order to investigate the relation between theta oscillations and STN firing rates, we simultaneously recorded intraoperative LFP and single unit activity from the STN during DBS surgery as a third group of participants performed an Eriksen flanker task. We found that theta oscillations entrain individual neurons of the STN during conflict, and that the theta locked neurons were associated with elevated firing rates during conflict. Taken as a whole our data yield insight into how theta oscillatory communication between the STN and the frontal cortex may modulate spiking activity in the STN thereby allowing the optimal behavioral response to be selected.

Disclosures: **B.A. Zavala:** None. **P. Brown:** None. **K. Zaghloul:** None.

Nanosymposium

587. Human Decision-Making: Neural Mechanisms

Location: 150A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 587.09

Topic: F.01. Human Cognition and Behavior

Support: FSU BQFW Initiative

Bial Foundation

Ralph Schlaeger Charitable Foundation

Mathers Foundation

Title: Predicting actions in speeded reaction-time and delayed-action tasks, an intracortical human study

Authors: *U. MAOZ[1,2], L. MUDRIK-DENAN[1,2], U. RUTISHAUSER[2,1], A. MAMELAK[2], C. KOCH[3];
[1]Caltech, Pasadena, CA; [2]Neurosurg., Cedars Sinai Med. Ctr., Los Angeles, CA; [3]Allen Inst. for Brain Sci., Seattle, WA

Abstract: What causal role consciousness plays in decision making is of great neuroscientific interest. The answer to this question even transgresses the boundaries of science: if consciousness plays no role in decision making, this challenges long-held notions of free will and moral responsibility. In recent decades, some neuroscientific research has shown that there is neural information about subjects' upcoming action long before they report having decided which action to carry out. Some have interpreted this as strong evidence against the causal role of consciousness in decision making. However, those studies focused on random decisions - such as raising the left or right hand with no reason, purpose or consequences - while the free will and moral responsibility debates center on deliberate decisions. What is more, it was shown that the timing that subjects report for the onset their decisions is highly inaccurate and systematically biased, and accordingly should not be used as a measure of decision onset. We previously demonstrated that, for deliberate decisions, information about upcoming actions could be increasingly well decoded from intracortical brain signals in a delayed-action task. Another study of ours also suggested that the action-predictive information before the reported decision time does not generalize well from random to deliberate decisions. We therefore focused on deliberate decisions in a competitive environment - a matching-pennies game where subjects won (lost) $0.10 if they won over (lost to) the computer in each trial. We did not rely on subjective reporting of decision onset. Instead, we asked our participants - consenting epilepsy patients implanted with intracortical electrodes for clinical purposes - to play two versions of the game. In one block they pressed the left or right button immediately upon deciding. In the other they first indicated when they made up their minds (by pressing both buttons) and then waited for a time-jittered go signal to indicate their choice with a button press. We compared the availability of predictive information about the content of upcoming decisions in the two versions. We found such predictive information mostly in the supplementary motor-area (SMA). Action-predictive activity in the SMA was mainly apparent just before action onset in the immediate-action blocks and just before the reported decision onset in the delayed-action blocks. Our results therefore do not support the existence of predictive information in the brain about upcoming action long before the onset of subject's conscious experience of having decided, at least for deliberate decisions.

Disclosures: U. Maoz: None. **L. Mudrik-Denan:** None. **U. Rutishauser:** None. **A. Mamelak:** None. **C. Koch:** None.

Nanosymposium

587. Human Decision-Making: Neural Mechanisms

Location: 150A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 587.10

Topic: F.01. Human Cognition and Behavior

Support: PhD-Scholarship by Konrad Adenauer Stiftung

Title: A task-independent neural representation of subjective certainty

Authors: *J. HEEREMAN[1,2], H. HEEKEREN[1,3,2];
[1]Freie Univ. Berlin, Berlin, Germany; [2]Berlin Sch. of Mind and Brain, Humboldt Universitaet, Berlin, Germany; [3]Dahlem Inst. for the Neuroimaging of Emotion, Freie Universitaet Berlin, Germany

Abstract: Am I really sure? This is a question not only scientists ask themselves but practically everybody everyday. De Gardelle & Mamassian (2014) provide behavioral evidence supporting the view that ones subjective confidence in a decision (i.e. the degree of belief subjects have in the correctness of their choice) is represented in a task-independent format. Previous neuroimaging studies identified neural correlates of decision confidence (e.g. Fleming et al. 2012). However, the generalizability of their results to situations where subjects rate percepts or objects, (e.g., how certain they are about a stimulus identity) and the dependence of this neural representation on a particular task remain unclear. Here, we therefore ask whether there is a task-independent neural representation of degrees of subjective certainty (i.e. a neural representation of subjective certainty that remains constant across two tasks). To address this question we used two perceptual decision tasks in combination with functional Magnetic Resonance Imaging (fMRI) (Siemens, 3T, TR=2). 22 participants performed a visual color/motion detection task. Subjects saw on the display a cloud of colored moving dots. In the beginning of each trial they were cued whether to attend motion and ignore color or attend color and ignore motion. Depending on the cue after stimulus presentation (750 ms) they had to rate their degree of certainty that motion was to the left or right or that there were more red dots than blue dots present (or more blue dots than red dots). In continuation they indicated the respective direction or color. All subjects completed 5 runs à 64 trials. Importantly, to exclude discriminability as confound we used one constant stimulus-intensity throughout the whole experiment. This intensity was calibrated to a predefined level of average subjective certainty (instead of performance). Using the ratings to parametrically modulate our main regressor we analysed the two trial-types (color and motion) separately. On the resulting contrasts we then performed conjunction analyses. For the negative parametric contrasts of certainty we found a significant

effect (conjunction null) in dorsal anterior cingulate cortex (DACC) (x=-6, y=11, z=49, MNI). That is, we found an activation in DACC that increases as certainty decreases and remains constant across two visual tasks. Importantly, due to the constant stimulus-intensity used this activation is independent of task-difficulty. In conclusion our data provide strong evidence for a task independent neural representation of subjective certainty.

Disclosures: J. Heereman: None. **H. Heekeren:** None.

Nanosymposium

587. Human Decision-Making: Neural Mechanisms

Location: 150A

Time: Tuesday, November 18, 2014, 1:00 PM - 3:45 PM

Presentation Number: 587.11

Topic: F.03. Motivation and Emotion

Support: ANR-11-EMCO-0011

LaBex Cortex

Title: Subjective confidence in one's decision and group size effect during group decisions

Authors: *S. A. PARK, S. GOIAME, J.-C. DREHER;
Reward and decision making group, Cognitive Neurosci. Ctr., Inst. Des Sci. Cognitives, CNRS, Bron, France

Abstract: A number of recent neuroimaging studies have investigated the neural underpinnings of the subjective confidence we have in our choices. Yet, it is still unknown how subjective confidence and choices interact at the levels of brain and behavior when confronted with others' opinions in a group. This is particularly true for group decisions made in ecological situation, such as sentencing a criminal within a jury, which can be affected by group size (jury). Here, we used fMRI to investigate the brain mechanisms underlying the adjustment of one's initial judgment confronted with the judgments of others during third-party punishment of a jury. We scanned 25 healthy subjects while they performed a jury decision task. Subjects first read a murder case describing the facts and circumstances. Half of the scenarios was designed to induce sympathy for the defendant, while the other half was non-sympathetic. Subjects made 3 successive ratings concerning each criminal case: (1) criminal sentence in prison years; (2) level of subjective confidence in their own judgment; (3) reconsideration of the first judgment after knowing the average prison years sentenced by the other jury. Importantly, we varied the group

size (jury=5 or 20) and examined how the brain integrates one's decision confidence with adjustment of the criminal sentence when confronted to a small or large jury, respectively inducing higher or smaller sense of responsibility. We compared two types of behavioral models to account for the reconsideration of one's own initial decision when confronted to different sizes of jury. The first model assumed that the jurors changed their judgment to conform to a social norm, while the second model used Bayesian inferences incorporating the difference between judgments, the subjective confidence in the first criminal sentence (metacognitive awareness in decisions) and the number of jurors in the group. We found that this Bayesian inference model explained better the reconsideration of the criminal sentence after knowing the judgment of the other members of the jury. At the brain level, the anterior cingulate response increased with the dissimilarity between one's own judgment and the others'. Moreover, among the brain regions more responsive to higher group size, the bilateral frontopolar cortex response strongly correlated with the individual differences in how much the jury considered the difference in distributions of judgments in terms of the group size when confronting the judgment of others. Together, our results show that during third-party legal decisions, specific brain regions integrate metacognitive awareness of one's choice and of the sense of responsibility.

Disclosures: S.A. Park: None. **S. Goiame:** None. **J. Dreher:** None.

Nanosymposium

670. Complex Neurodegenerative Pathologies

Location: 140A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:00 AM

Presentation Number: 670.01

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

Support: NIH/NIA Grant R41AG043243

ADDF Grant 20121209

Title: Discovery of a dual therapeutic approach for preventing or slowing the progression of Alzheimer's disease (AD)

Authors: *J. A. SCHETZ[1,2], S. KIM[1], A. M. TUTTLE[1], D. DALWADI[1], C. BORSAN[3], G. BEATON[3], S. B. RAVULA[3], F. C. TUCCI[3];
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Abstract: No effective disease-modifying treatments currently exist for Alzheimer's disease (AD) and without such treatments, the number of individuals afflicted and the associated financial and caregiver burdens will increase dramatically. To address this unmet medical and societal need, our research is focused on the development of drug treatments capable of preventing or slowing the progression of AD. Brain inflammation triggered by chronic stress is a proven component in the pathogenic cascade leading to mild cognitive impairment (MCI) and AD. When the reactive inflammatory molecules nitric oxide and superoxide are in surplus, they combine to form the brain-impairing reactive species peroxynitrite. This perpetuates inflammation thereby leading to the progressive neurodegeneration seen in AD. Our dual strategy for treating AD includes interrupting the cycle of peroxynitrite production and enhancing resilience to inflammatory brain insults. The former will be achieved by selectively blocking the unsafe elevation of nitric oxide, a molecule required to form peroxynitrite. The latter will be achieved by facilitating the secretion of brain-derived neurotrophic factor (BDNF) which will promote neurogenesis and strengthen synapses; cognitive decline is slower in AD patients with higher levels of brain BDNF, while lower levels are associated with an increased risk of AD. When brain cells experience high levels of inflammatory (oxidative) stress, the Sigma-1 receptor (S1R) protein is capable of regulating nitric oxide levels and mediating BDNF secretion. For this reason, we are discovering and developing drugs that target the S1R. By targeting functionally selective signaling pathways measured with a unique in vitro high thoughput platform, we have engineered small molecules that both reduce nitric oxide levels in response to an AD-type stressor and increase secretion of BDNF in neuronal and glial cells. Our drug discovery efforts have lead to the identification of a novel class of orally-active compounds, exemplified by EPGN296, which we have optimized for CNS drug-like properties.

Disclosures: J.A. Schetz: None. **S. Kim:** None. **A.M. Tuttle:** None. **D. Dalwadi:** None. **C. Borsan:** None. **G. Beaton:** None. **S.B. Ravula:** None. **F.C. Tucci:** None.

Nanosymposium

670. Complex Neurodegenerative Pathologies

Location: 140A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:00 AM

Presentation Number: 670.02

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

Support: NIA

Title: CypD deficiency attenuates mitochondrial and synaptic dysfunction and cognitive decline in STZ-induced type 1 diabetic mouse model

Authors: *L. WU, S. YAN, F. DU, J. VANGAVARAGU, S. YAN;
Pharmacol. & Toxicology, Univ. of Kansas, Lawrence, KS

Abstract: Abstract Diabetes mellitus is a heterogeneous metabolic disorder associated with an increased risk of synaptic injury and cognitive dysfunction. Diabetic patients have higher incidence of dementia including the Alzheimer's type. However, the underlying mechanisms of diabetes-associated cognitive deficits remain to be elucidated. Here we demonstrated that Cyclophilin D (CypD)-mediated mitochondrial permeability transition pore (mPTP) contributed to diabetic mitochondrial abnormalities and related synaptic and cognitive dysfunction using streptozotocin (STZ)-induced type 1 diabetic mouse model. The absence of CypD reversed synaptic dysfunction as shown by the attenuation in the reduction of long-term potentiation (LTP) in the hippocampus of STZ mice. Notably, compared with STZ treated nontransgenic mice, CypD-deficient STZ mice exhibited substantial improvement in learning and memory function in Morris Water Maze navigation task. The potential mechanisms of reversing cognitive deficits by blocking CypD might be reducing oxidative stress-induced damage on mitochondrial respiratory function. Our results provide new insights into the role of CypD-dependent mitochondrial mPTP in brain mitochondrial malfunction, synaptic perturbation and cognitive impairment. Therefore, targeting CypD could be a potential therapeutic strategy for diabetes-associated cognitive dysfunction and dementia including Alzheimer's disease.

Disclosures: L. Wu: None. **S. Yan:** None. **F. Du:** None. **J. Vangavaragu:** None. **S. Yan:** None.

Nanosymposium

670. Complex Neurodegenerative Pathologies

Location: 140A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:00 AM

Presentation Number: 670.03

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

Support: NIH grant AG032297

Title: Chronic oxotremorine treatment ameliorates depressive phenotype in a rodent model of Alzheimer's disease

Authors: *D. V. NAIR, M. M. AL-BADRI, H. PENG, N. SCHENKMAN, J. PACHECO-QUINTO, C. B. ECKMAN, D. IACONO, E. A. ECKMAN;
Atlantic Hlth. Systems, Morristown, NJ and Biomed. Res. Inst. of New, Cedar Knolls, NJ

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative condition that is characterized by changes to brain structure and function. It is estimated that depression and other neuropsychiatric symptoms occur in up to 90% of AD patients, yet the neurobiological basis of these symptoms and their influence on the clinical course of AD remain unclear. Using a rat model of AD-like basal forebrain cholinergic cell loss, our lab has previously shown that central administration of a muscarinic receptor agonist, oxotremorine, for 4 weeks could induce hippocampal neurogenesis and reverse the spatial working memory deficit triggered by cholinergic denervation. Preliminary experiments conducted with this model in our lab also revealed a depressive phenotype emerging between 11 and 15 weeks after cholinergic denervation. The depressive phenotype was detected using a sucrose consumption test and further confirmed by forced swim test. The goal of the present study was to determine whether effects of chronic oxotremorine treatment could ameliorate the depressive phenotype observed after selective cholinergic cell loss in the basal forebrain. Adult female Sprague Dawley rats were injected intracerebroventricularly (icv) with the immunotoxin 192-IgG-saporin (SAP), to induce AD-like basal forebrain cholinergic cell loss. After a 5 week recovery period, the rats then received 8 weeks of icv infusion of either oxotremorine or vehicle (saline) via osmotic minipump. Behavioral testing to assess the depressive phenotype was carried out using the sucrose consumption test every 2 weeks during oxotremorine treatment. The phenotype was further confirmed by forced swim test. Biochemical analysis of a range of markers including tryptophan hydroxylase, the rate limiting enzyme for synthesis of serotonin, was performed after extraction of the brains following the behavioral tests. Results of these experiments demonstrate that oxotremorine treatment prevents the development of the depressive phenotype in SAP-lesioned rats. A number of oxotremorine-treated rats showed increases in tryptophan hydroxylase, suggesting a possible mechanism for the improved behavioral phenotype Based on these data, we propose that 192-IgG saporin lesioned rats may be an effective model for studying the pathophysiology and therapeutic modulation of age- and neurodegeneration-related neuropsychiatric symptoms such as depression.

Disclosures: D.V. Nair: None. **M.M. Al-Badri:** None. **H. Peng:** None. **N. Schenkman:** None. **J. Pacheco-Quinto:** None. **C.B. Eckman:** None. **D. Iacono:** None. **E.A. Eckman:** None.

Nanosymposium

670. Complex Neurodegenerative Pathologies

Location: 140A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:00 AM

Presentation Number: 670.04

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

Support: NS057295

Title: Hedgehog antagonist, Cyclopamine, inhibits γ-secretase-mediated cleavage of APP by altering retrograde trafficking of endocytosed APP C-terminal fragments

Authors: *A. G. VOROBYEVA[1], R. LEE[2], S. MILLER[2], P. KANDELWAL[2], G. DISTEFANO[2], A. GANGEMI[2], D. MARENDA[2], A. SAUNDERS[2];
[1]Biol., [2]Drexel Univ., Philadelphia, PA

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease leading to memory loss. Numerous lines of evidence suggest that Aβ, a neurotoxic peptide, initiates a cascade that ultimately results in synaptic dysfunction and eventually neuronal death. Aβ is generated from Amyloid Precursor Protein (APP) by the proteolytic process of β- and γ-secretases. Using an in vitro model we observed an increase in APP-CTFs and a decrease in Aβ and AICD upon cyclopamine treatment. Biochemical and microscopy analysis revealed cyclopamine increased APP endocytosis and accumulation of APP-CTF-positive puncta was observed. The aforementioned puncta colocalized with lysosomal markers while decreased colocalization with trans-Golgi network markers was observed. Our data suggests cyclopamine redirects retrograde trafficking of APP-CTFs to the lysosomal degradation pathway thereby decreasing trafficking to trans-Golgi network for γ-secretase proteolysis and Aβ generation. Finally, we confirmed cyclopamine ameliorates the effects of neurotoxic Aβ peptide using our transgenic Drosophila model of AD. Taken together, our data suggests cyclopamine is a novel regulator of APP metabolism in vitro and in vivo.

Disclosures: A.G. Vorobyeva: None. **R. Lee:** None. **S. Miller:** None. **P. Kandelwal:** None. **G. DiStefano:** None. **A. Gangemi:** None. **D. Marenda:** None. **A. Saunders:** None.

Nanosymposium

670. Complex Neurodegenerative Pathologies

Location: 140A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:00 AM

Presentation Number: 670.05

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

Support: NUHS Seed Grant for Basic Science

Title: Regulation of brain insulin signaling by apolipoprotein E in a mouse model of Alzheimer's disease

Authors: *B.-S. WONG, E. CHAN;
Natl. Univ. of Singapore, Singapore, Singapore

Abstract: Human apolipoprotein E4 (hApoE4) is a major genetic risk factor for Alzheimer's disease (AD), but it is unclear how harbouring hApoE4 causes earlier disease manifestation. We have recently reported a hApoE isoform-dependent response to brain insulin signalling. Mice with targeted replacement for hApoE4 have impaired brain insulin signalling and lower insulin levels as compared to hApoE3 mice. To examine the connection between hApoE and brain insulin signaling in AD, we have crossed our mice carrying familial-AD mutant human amyloid precursor protein (hAPP[FAD]) with the hApoE3 and hApoE4 mice. Our results show a novel association between the hApoE with the insulin receptor (IR) and Aβ. In the hApoE3/hAPP[FAD] mouse brain, hApoE has increased association with IR. In hApoE4/hAPP[FAD] mouse brain, hApoE has increased binding with Aβ. This ApoE genotype-dependent interaction with IR occurs prior to detectable change in brain insulin signaling and cognitive performance. The absence of hApoE4 binding to IR can lead to brain insulin signaling impairment and is linked to higher amyloid level and greater cognitive decline.

Disclosures: B. Wong: None. **E. Chan:** None.

Nanosymposium

670. Complex Neurodegenerative Pathologies

Location: 140A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:00 AM

Presentation Number: 670.06

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

Support: Supplememnt to NIH Grant NS079637

Title: Cerebrovascular and Alzheimer's pathologies in mouse models of mixed dementia

Authors: *H. M. BROTHERS[1], C. LATTA[1], T. L. SUDDUTH[1], K. BRAUN[1], E. M. WEEKMAN[1,2], D. M. WILCOCK[1,2];
[1]Sanders-Brown Ctr. on Aging, Sanders-Brown Ctr. On Aging, Lexington, KY; [2]Physiol., Univ. of Kentucky, Lexington, KY

Abstract: Dementia is not defined by a single cause or pathology, and is most often attributed to Alzheimer's disease (~70%) or vascular dementia (~17%), yet a considerable number of dementia cases (20-50%) share aspects of both and are better characterized as mixed dementia. Better understanding of dementias within this spectrum can improve diagnosis, inform interpretation of clinical data confounded by co-morbidity, and direct therapeutic approaches. To study the commonalities and differences between these forms of dementia, we compared and combined aspects of cerebrovascular disease (chronic cerebral hypoperfusion) and Alzheimer's disease (transgene-driven tau or amyloid pathology). We induced chronic cerebral hypoperfusion by wrapping each common carotid artery with a microcoil that remains in situ and reduces cerebral blood flow to approximately 80%; a procedure called bilateral carotid artery stenosis (BCAS). To create conditions of mixed dementia, we performed BCAS in two transgenic models of AD pathology; the rTg4510 which overexpresses mutant human tau, and APP/PS1 that develops ß-amyloid pathology. Transgenic mice and their wildtype littermates received BCAS or sham surgeries in adulthood near the onset of pathology for their respective genotype (rTg4510 at 2 months or APP/PS1 at 6 months) or during advanced pathology (rTg4510 at 7 months or APP/PS1 at 11 months) and survived short-term chronic cerebral hypoperfusion for 1 month. Another set underwent surgery at the onset of pathology and survived long-term chronic cerebral hypoperfusion for 6 months. We evaluated spatial memory impairment and gross motor performance. We characterized the neuroimmune phenotype, white matter integrity, tau pathology and ß-amyloid pathology through biochemical and histological analysis. The progression of memory impairment and disease pathology is altered in the mixed dementia models, and these changes are relative to the age of onset and duration of hypoperfusion. For example, there is an interaction in spatial memory performance in which hypoperfusion has a larger impact on impairment in aged APP/PS1 mice. These findings highlight changes in the time-course of cognitive impairment and pathology that might be expected in the substantial clinical population with co-morbid vascular and Alzheimer's dementias.

Disclosures: H.M. Brothers: None. **C. Latta:** None. **T.L. Sudduth:** None. **K. Braun:** None. **E.M. Weekman:** None. **D.M. Wilcock:** None.

Nanosymposium

670. Complex Neurodegenerative Pathologies

Location: 140A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:00 AM

Presentation Number: 670.07

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

Support: NIH 5F31NS078896-02

NIH 1R01NS082672-01A1

NIH 2P50AG016574-16

Mayo Clinic

University of Florida

Title: The Parkinson's Disease-related protein LRRK2 is a novel tau kinase

Authors: *J. M. LEWIS[1], R. M. BAILEY[2], J. COVY[3], M. J. HAMM[2], D. W. DICKSON[4], B. I. GIASSON[2];
[1]Dept Neurosci., [2]Univ. of Florida, Gainesville, FL; [3]Stanford, Palo Alto, CA; [4]Mayo Clin., Jacksonville, FL

Abstract: Although tau pathology is found in Alzheimer's Disease (AD), it also can be found as a secondary pathology in Parkinson's disease (PD). Other lines of circumstantial evidence have previously linked tau with PD and a proven link between AD and PD would be particularly exciting as it could open therapeutic avenues that could address both diseases. Given that tau is regulated heavily in both normal and disease states by kinases, we sought to determine if the PD-linked kinase LRRK2 (leucine-rich repeat kinase 2) may be a novel tau kinase which could influence development of tauopathy and underlie the link between these diseases. We performed a series of experiments which combined in vitro, cell culture and novel mouse modeling studies to demonstrate that LRRK2 enhances tau phosphorylation profiles as well as tau pathology and neurodegeneration. Using MS, we identified a number of epitopes in tau that appeared to be phosphorylated by LRRK2. Many of these sites were epitopes that had been minimally explored in the tau field including pT149 and pT153. Further, we crossed mice expressing moderate levels of LRRK2 with the rTg4510 tau transgenic model of tauopathy and demonstrated that LRRK2 enhanced the phosphorylation and insolubility of the tau protein. We have now followed those studies to demonstrate that this interaction also increases the neurodegeneration observed in the LRRK2/rTg4510 mice when compared to rTg4510 mice alone. We have also recently identified multiple physiologically-relevant factors that either increase or block the ability of LRRK2 to target tau and these data will be presented. We have demonstrated that LRRK2 can directly phosphorylate tau and this interaction likely underlies the increased tauopathy observed in LRRK2/rTg4510 mice compared to the tau transgenic model alone. We have now explored additional minimally investigated phospho-epitopes in tau and defined critical physiological manipulations that dramatically increased or completely block phosphorylation of tau by LRRK2. This link could provide a new avenue of research for therapy development to target not only Parkinson’s disease but also the broader group of tauopathies such as Alzheimer’s Disease.

Disclosures: **J.M. Lewis:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Mayo Clinic royalties. **R.M. Bailey:** None. **J. Covy:** None. **M.J. Hamm:** None. **D.W. Dickson:** None. **B.I. Giasson:** None.

Nanosymposium

670. Complex Neurodegenerative Pathologies

Location: 140A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:00 AM

Presentation Number: 670.08

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

Support: NIA Grant R37AG037319

Title: Oxidative stress-mediated activation of extracellular signal-regulated kinase contributes to mild cognitive impairment-related mitochondrial dysfunction

Authors: X. GAN[1,2], L. WU[1], S. HUANG[1,2], F. DU[1], C. ZHONG[1], Y. WANG[1], H. YU[2], R. H. SWERDLOW[3], J. X. CHEN[4], *S. YAN[1];
[1]Univ. of Kansas, Lawrence, KS; [2]Sichuan Univ., Chengdu, China; [3]Univ. of Kansas Med. Ctr., Kansas City, KS; [4]Mem. Sloan-Kettering Cancer Ctr., New York, NY

Abstract: Mild cognitive impairment (MCI) occurs during the pre-dementia stage of Alzheimer's disease (AD) and is characterized by a decline in cognitive abilities that frequently represents a transition between normal cognition and AD dementia. Its pathogenesis is not well understood. Here, we demonstrate the direct consequences and potential mechanisms of oxidative stress, mitochondrial dynamic and functional defects in MCI-derived mitochondria. Using cytoplasmic hybrid (cybrid) cell model in which mitochondria from MCI or age-matched non-MCI subjects were incorporated into a human neuronal cell line depleted of endogenous mitochondrial DNA, we evaluated the mitochondrial dynamics and function, as well as the role of oxidative stress in the resultant cybrid lines. We demonstrated increased expression levels of mitofusin 2 (Mfn2) is markedly induced by oxidative stress in MCI-derived mitochondria along with aberrant mitochondrial functions. Inhibition of oxidative stress rescues MCI-impaired mitochondrial fusion/fission balance as shown by the suppression of Mfn2 expression, attenuation of abnormal mitochondrial morphology and distribution, and improvement in mitochondrial function. Furthermore, blockade of MCI related stress mediated activation of extracellular signal-regulated kinase (ERK) signaling not only attenuates aberrant mitochondrial morphology and function but also restores mitochondrial fission and fusion balance, in particular

inhibition of overexpressed Mfn2. Our results provide new insights into the role of the oxidative stress-ERK-Mfn2 signal axis in MCI-related mitochondrial abnormalities, indicating that the MCI phase may be targetable for the development new therapeutic approaches that improve mitochondrial function in age-related neurodegeneration.

Disclosures: **X. Gan:** None. **L. Wu:** None. **S. Huang:** None. **F. Du:** None. **C. Zhong:** None. **Y. Wang:** None. **H. Yu:** None. **R.H. Swerdlow:** None. **J.X. Chen:** None. **S. Yan:** None.

Nanosymposium

671. Alzheimer's Disease: Experimental Therapeutics I

Location: 147B

Time: Wednesday, November 19, 2014, 8:00 AM - 10:00 AM

Presentation Number: 671.01

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

Support: Health Research Council of New Zealand

Department of Physiology, University of Otago

Title: Effects and mechanism of action of estren on beta amyloid-induced cholinergic and behavioural deficits

Authors: *A. KWAKOWSKY[1], K. POTAPOV[1], S. KIM[1], K. PEPPERCORN[2], W. P. TATE[2], I. M. ABRAHAM[3];
[1]Ctr. for Neuroendocrinology and Dept. of Physiol., [2]Dept. of Biochem., Univ. of Otago, Dunedin, New Zealand; [3]Dept. of Physiol., Univ. of Pecs, Pecs, Hungary

Abstract: Deatrimental side effects of estrogen replacement therapy have increased efforts to develop compounds that selectively reproduce beneficial estrogen actions and one such compound is estren (4-estren-3α, 17β-diol). Estren is a selective non-classical estrogen like signaling activator with neuroprotective effects in vitro. Alzheimer`s disease (AD) is characterized by accumulation of neurotoxic beta-amyloid (Aβ) and impaired cognitive function linked to early loss of cholinergic neurons. In this study, we have examined the effects and mechanism of action of estren treatment on Aβ1-42-induced cholinergic neurotoxicity and behavioural deficit in vivo. Evaluation of adult female wild-type mice that received unilateral Aβ1-42 injection into the nucleus basalis magnocellularis complex (NBM) of the basal forebrain showed 30 % decrease in cholin-acetyltransferase (ChAT)-immunoreactive cell bodies in the NBM and acetylcholinesyterase (AChE)-stained fibers in the somatosensory cortex of the

lesioned hemisphere. A single injection of 0.33 ng/g estren 1 h after Aβ1-42 administration did not have an effect on cholinergic cell loss in the NBM, but it restored the ipsilateral cholinergic fiber density in the somatosensory cortex. Mice that received bilateral injection of Aβ1-42 into the NBM demonstrated impaired learning skills compared to control groups. However, a single 33 ng/g estren treatment was able to restore the deficits of learning behaviours. We have previously reported that estradiol rapidly induces extracellular-signal-regulated kinase 1 and 2 (ERK1/2) and cAMP response element binding protein (CREB) phosphorylation in cholinergic neurons in vivo. In the present study, we found that administration of estren to adult female mice resulted in significantly increased phosphorylation of ERK1/2 and CREB in cholinergic neurons of the NBM within 30 min. Interestingly, phosphorylation of ERK1/2 was significantly increased at the Aβ1-42 treated brain side compared to non-treated side after estren administration. In summary, these findings indicate that estren might hold potential as a molecular target for AD prevention and treatment.

Disclosures: **A. Kwakowsky:** None. **K. Potapov:** None. **S. Kim:** None. **K. Peppercorn:** None. **W.P. Tate:** None. **I.M. Abraham:** None.

Nanosymposium

671. Alzheimer's Disease: Experimental Therapeutics I

Location: 147B

Time: Wednesday, November 19, 2014, 8:00 AM - 10:00 AM

Presentation Number: 671.02

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

Support: CIHR

Title: UCHL1 inhibits app processing and delays alzheimer progression *in vivo*

Authors: *M. ZHANG, F. CAI, S. ZHANG, S. ZHANG, W. SONG;
Dept. of Psychiatry, The Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Deposition of Amyloid β protein (Aβ) to form neuritic plaques in the brain is one of the pathological features of Alzheimer's disease (AD). Aβ is produced from amyloid β precursor protein (APP) by β-secretase BACE1 and γ-secretase complex. The abnormal accumulation of Aβ initiates neuronal dysfunction and plays an important role in AD pathogenesis. Ubiquitin carboxyl-terminal hydrolase L1 (UCHL1) is a de-ubiquitinating enzyme that cleaves ubiquitin at its carboxyl terminal. Dysfunction of UCHL1 has been reported in various neurodegenerative diseases. UCHL1 protein level is reduced in AD and is inversely proportional to the number of

neurofibrillary tangles in AD brains. However, whether UCHL1 affects Aβ production and AD progression remains unknown. Previously we discovered that BACE1 is degraded through the ubiquitin-proteasome pathway and that UCHL1 accelerates BACE1 degradation. Here we demonstrated that intracranial injection of UCHL1-expressing AAV reduced Aβ production, inhibited amyloid plaque formation and rescued memory deficits in APP23/PS45 double transgenic mice. Moreover, UCHL1 regulated Aβ production in vivo mainly by reducing APP protein level. We further revealed in multiple cell lines that UCHL1 accelerated the lysosomal degradation of APP by promoting its ubiquitination. Our results showed that APP protein level may be reduced by direct interaction with UCHL1, or by the increased free ubiquitin level regulated by UCHL1. Taken together, our study suggested that UCHL1 may delay AD progression by regulating APP degradation, and that overexpression of UCHL1 may be a safe and effective approach to treat AD.

Disclosures: **M. Zhang:** None. **F. Cai:** None. **S. Zhang:** None. **S. Zhang:** None. **W. Song:** None.

Nanosymposium

671. Alzheimer's Disease: Experimental Therapeutics I

Location: 147B

Time: Wednesday, November 19, 2014, 8:00 AM - 10:00 AM

Presentation Number: 671.03

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

Support: NSFC 81271424

NSFC 31171313

Title: Lamotrigine attenuates Alzhemer's pathology in app/ps1 transgenic mice

Authors: *Q. MA;
Inst. of neuroscience, Soochow Univ., Jiangsu, China

Abstract: Hyperactivity and its compensatory mechanisms may causally contribute to synaptic and cognitive deficits in Alzheimer’s disease. Blocking the hyperactivity of neural network, could prevent synaptic and cognitive deficits in human APP transgenic mice. In this study, we show a beneficial role of Lamotrigine (LTG) in APP/PS1 transgenic mice. The numbers of amyloid plaques, the loss of spines and suppression of long-term potentiation are reduced in APP/PS1 treated with LTG. The effects of LTG eventually attenuate deficits in learning and

memory of APP/PS1 mice. An reduction of frequency of abnormal spikes and levels of BACE1, while an upregulation of levels brain-derived growth factor (BDNF) and nerve growth factor (NGF) have been observed in LTG-treated APP/PS1 mice. Therefore, these observations demonstrate that LTG attenuates AD pathology may through multiple mechanisms.

Disclosures: Q. Ma: None.

Nanosymposium

671. Alzheimer's Disease: Experimental Therapeutics I

Location: 147B

Time: Wednesday, November 19, 2014, 8:00 AM - 10:00 AM

Presentation Number: 671.04

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

Support: NIH Grant AG032784

NIH Grant ES016774

NIH Grant AG0428

Title: The case for using a papain-like cysteine protease inhibitor as an Alzheimer's disease therapeutic

Authors: *G. R. HOOK[1], S. JACOBSEN[1,2], M. KINDY[3,4], V. HOOK[5];
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Abstract: Disease-modifying therapeutics for Alzheimer's disease (AD) are lacking despite considerable efforts to develop such. This presentation makes the case that inhibitors of the papain-like cysteine proteases (PCP), especially cathepsin B (CatB) and calpain, are excellent AD drug candidates. The PCP inhibitor, E64d (aka L-*trans*-epoxysuccinyl(OEt)-Leu-3methylbutlamide, EST, and loxistatin) is a tool compound, which has demonstrated preclinical proof-of concept for the effectiveness of this therapeutic approach. Data in animal models of AD and related neurodegenerative diseases show that E64d treatment improves memory deficits and brain pathology, prevents neuronal cell death, and lowers neuroinflamatory processes. E64d and structurally related tool inhibitor compounds have been shown to (a) reduce neurotoxic brain amyloid-β (Aβ), both full-length Aβ(1-40/42) and the pernicious pyroglutamate Aβ(3-40/42) species, (b) reduce the proapoptotic cell death biomarkers of Bax, Bid cleavage, cytochrome c,

and caspase 3 activation, and (c) reduce the proinflammatory cytokines such as IL-6 and IL-1β whilst (d) increasing neuroprotective proteins like APPα and Bcl-2. Importantly, E64d has been safely used in man and shown to have a wide therapeutic window for the safe treatment of a different indication, muscular dystrophy. Proprietary E64d derivatives having improved pharmaceutical properties, including better efficacy than E64d, will be advanced to the clinic where they are expected to have similar therapeutic and safety-toxicological profile as E64d. PCP inhibitors are an exciting new class of AD therapeutics, and likely will provide multiple beneficial effects and can be safely tested in man.

Disclosures: G.R. Hook: A. Employment/Salary (full or part-time):; salary, American Life Science Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Equity interest, American Life Science Pharmaceuticals. **S. Jacobsen:** A. Employment/Salary (full or part-time):; Salary, AstraZeneca. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Equity interest, American Life Science Pharmaceuticals. **M. Kindy:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Equity interest, Applied Neurotechnology. **V. Hook:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Equity interest, American Life Science Pharmaceuticals.

Nanosymposium

671. Alzheimer's Disease: Experimental Therapeutics I

Location: 147B

Time: Wednesday, November 19, 2014, 8:00 AM - 10:00 AM

Presentation Number: 671.05

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

Support: NIH grant NS079637 (DMW)

Title: The plaque specific antibody mE8c initiates a robust, M2b polarized neuroinflammatory response with enhanced beta-amyloid clearance relative to the N-terminal antibody 3D6

Authors: *T. L. SUDDUTH[1], E. M. WEEKMAN[2], M. M. RACKE[3], J. T. HOLE[3], J. A. TZAFERIS[3], R. B. DEMATTOS[3], D. M. WILCOCK[2];
[1]Sanders Brown Ctr. On Aging, Univerisity of Kentucky, Lexington, KY; [2]Univ. of Kentucky, Lexington, KY; [3]Eli Lilly and Co., Indianapolis, IN

Abstract: The plaque specific antibody mE8c recognizes the pyroglutamate Aβ species Aβp3-42. It has been shown in previous studies that the mE8c results in enhanced plaque clearance compared to a typical N-terminal monoclonal anti-Aβ antibody, specifically 3D6. In addition, mE8c results in enhanced phagocytosis by microglial cells in an ex vivo study. In the current study we examined the specific neuroinflammatory changes occurring as a result of mE8c and 3D6. PDAPP transgenic mice aged 21 months were passively immunized with 3D6, mE8c or an IgG2a isotype control. We performed gene expression analysis for genes categorizing inflammatory states termed M1 and M2. M2 can be further categorized as M2a, M2b and M2c. We also performed ELISA analysis of Aβ proteins on the same brain samples and histological analysis of brain amyloid load. We have found that the mE8c antibody promotes a robust M2b neuroinflammatory phenotype. This was characterized by high IL-10, low IL-12, and increased expression of Fcγ receptor, IL-1β and TNFα. The M2b response mediated by the mE8c antibody was significantly greater than that induced by the 3D6 antibody. Along with the robust neuroinflammatory change was a reduced Aβ load. An M2b phenotype appears to be beneficial in the clearance of Aβ from the brain without causing microhemorrhage as previously reported and we are exploring the mE8c antibody to determine why the antibody produces a robust M2b response in the brain.

Disclosures: **T.L. Sudduth:** None. **E.M. Weekman:** None. **M.M. Racke:** A. Employment/Salary (full or part-time):; Eli Lilly. **J.T. Hole:** A. Employment/Salary (full or part-time):; Eli Lilly. **J.A. Tzaferis:** A. Employment/Salary (full or part-time):; Eli Lilly. **R.B. DeMattos:** A. Employment/Salary (full or part-time):; Eli Lilly. **D.M. Wilcock:** None.

Nanosymposium

671. Alzheimer's Disease: Experimental Therapeutics I

Location: 147B

Time: Wednesday, November 19, 2014, 8:00 AM - 10:00 AM

Presentation Number: 671.06

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

Support: NIH AT006816 (GMC)

VA Merit (GMC, SAF)

Mary S Easton Consortium (GMC, SAF)

Title: Tau deletion exacerbates age-dependent deficits in the hippocampus and substantia nigra and is corrected by DHA and ALA supplementation

Authors: *Q.-L. MA[1,2], X. ZUO[1,2], F. YANG[1,2], E. TENG[1,2], S. FRAUTSCHY[1,2], G. COLE[1,2]; [1]UCLA, Los Angeles, CA; [2]GRECC (VA), Los Angeles, CA

Abstract: Hyperphosphorylation and accumulation of tau aggregates are prominent features in tauopathies, including Alzheimer's disease (AD). Although a toxic gain of function caused by tau accumulation is well-established, the contribution resulting a loss of tau function on synaptic and cognitive deficits remains poorly understood. Tau knockout (KO) and C57bl Mice were maintained on a breeder chow (5015, 11% fat, which lacks fishmeal, and thus DHA). One cohort was fed DHA, DHA+ALA or standard diet, for five months starting at (14-15 months old; n=8 to 9). In a separate cohort, age dependent effects on motor deficits and tyrosine hydroxylase were assessed in a separate cohort at 2-3 months, 8-9 months, 11-12 moths and 21-months (n=5 to 9). We report that old (19-20 months old, OKO) but not middle-aged (8-9 months old, MKO) tau KO mice develop Morris Water Maze (MWM) deficits and loss of hippocampal acetylated α-tubulin and excitatory synaptic proteins. Mild motor deficits and reduction in tyrosine hydroxylase (TH) in the substantia nigra were present by middle age but were not sufficient to cause MWM deficits, while OKO mice showed MWM deficits paralleling hippocampal deficits. Deletion of tau, a microtubule-associated protein (MAP), resulted in increased levels of MAP1A, MAP1B and MAP2 in MKO followed by loss of MAP2 and MAP1B in OKO. Hippocampal synaptic deficits in OKO mice were partially corrected with dietary supplementation with docosahexaenoic acid (DHA), and both MWM and synaptic deficits were fully corrected by combining DHA with α-lipoic acid (DHA/ALA), which also prevented TH loss. DHA or DHA/ALA restored phosphorylated and total GSK3β and attenuated hyperactivation of the tau C-Jun N-terminal kinases (JNKs), while increasing MAP1B, dephosphorylated (active) MAP2 and acetylated α-tubulin, suggesting improved microtubule stability and maintenance of active compensatory MAPs. Our mice are on an 11% fat diet (PMI5015) that contains DHA precursor alpha linolenic acid that lacks pre-formed DHA. Typical Western diets are 35% fat and low in DHA. Use of a 5% fat diet with DHA from fish meal (PicolabDiet 20) may explain a report of no deficits in aging tau KO (Morris et al, 2013). This suggests that widely used low fat/ fish meal supplemented rodent diets may be inadvertently therapeutic. Our results implicate the loss of MAP function in age-associated hippocampal deficits and identify a safe dietary intervention, rescuing both MAP function and TH in OKO mice. Therefore, in addition to microtubule-stabilizing therapeutic drugs, preserving or restoring compensatory MAP function may be a useful new prevention strategy.

Disclosures: Q. Ma: None. **X. Zuo:** None. **F. Yang:** None. **E. Teng:** None. **S. Frautschy:** None. **G. cole:** None.

Nanosymposium

671. Alzheimer's Disease: Experimental Therapeutics I

Location: 147B

Time: Wednesday, November 19, 2014, 8:00 AM - 10:00 AM

Presentation Number: 671.07

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

Support: A Grant-in-Aid for the Cooperative Research Project from Institute of Natural Medicine, University of Toyama in 2014

Title: DR-induced cognitive enhancement in Alzheimer's disease model mice, 5xFAD, and underlying mechanisms

Authors: ***Z. YANG**, T. KUBOYAMA, C. TOHDA;
Div.of Neuromedical Sci., Instit of Natural Medicine, Univ. of Toyama, Toyama, Japan

Abstract: Alzheimer's disease (AD) is a chronic progressive neurodegenerative disorder. Current therapies for AD are employed for symptomatic improvement and unfortunately insufficient to regain memory function. We consider axon/synapse formation activity is critical for the fundamental therapies of AD. Our previous studies have shown that the improvable activity against Aβ(25-35)-induced axonal atrophy in cultured cortical neurons correlates well to the effect on momory recovery in AD model mice. We found previously that the water extract of DR* (1, 10 μg/ml) significantly increased axonal density in cortical neurons when adminstered 4 days after Aβ(25-35) treatment. This study aim to clarify in vivo activity of DR and its mechanism. DR was adminstered to normal mice (ddY, male, 6 weeks old), and object recognition memory, object location memory test were carried out with 48-h interval time. DR (500 mg/kg, 7 days, p.o.) administration significantly enhanced object recognition and location memory. Using Alzheimer's disease model mice 5xFAD, memory improvement activity was investigated. DR (5, 50 and 500 mg/kg, p.o.) was administrated to 5xFAD mice for 21-31 days. Object recognition memory (1-h interval time), object location memory (1-h interval time) and episodic memory (10-min interval time each) were significantly improved by DR treatment dose-dependently. These results suggest that DR might be a promising drug to treat AD. We are now identifying major signal pathways of DR, using drug affinity responsive target stability (DARTS) method, direct binding proteins of DR extract were explored in mouse cortical lysate (ddY, male, 7 weeks old) by DARTS and 2D-PAGE methods, at least 16 candidates as target proteins were obtained. Further more, in order to clarify the active constituents in DR, bio-assay guided isolation was performed using subfractions of the extract. *A name of the crude drug is not open due to patent matters.

Disclosures: Z. Yang: None. **T. Kuboyama:** None. **C. Tohda:** None.

Nanosymposium

671. Alzheimer's Disease: Experimental Therapeutics I

Location: 147B

Time: Wednesday, November 19, 2014, 8:00 AM - 10:00 AM

Presentation Number: 671.08

Topic: C.19. Drug Discovery and Development

Support: Merck &Co

Title: Pharmacological inhibition or genetic deletion of both BACE1 and BACE2 results in hypopigmentation in the mouse

Authors: *M. E. KENNEDY[1], L. A. HYDE[2], L. STAHL[2], M. SMITH[3], D. LEVITAN[4], Y. LIN[2], B. WEIG[5], L. CHEN[3], K. BECKER[6], S. WHITE-HUNT[6], R. L. PEIFFER[7], B. A. MATTSON[7], B. CHEEWATRAKOOLPONG[2], T. WISNIEWSKI[2], M. KAVANA[2], R. TERRY[5], K. TANIS[8], J. SCOTT[9], J. CUMMING[9], E. M. PARKER[2];
[1]Merck Res. Labs, Boston, MA; [2]Pharmacol., [3]Mol. Biomarkers, [4]Discovery Pharmacogenomics, [5]Neurosci., [6]Safety Assessment and Lab. Animal Resources, Merck Res. Labs, Kenilworth, NJ; [7]Safety Assessment and Lab. Animal Resources, [8]Discovery Pharmacogenomics, Merck Res. Labs, West Point, PA; [9]Medicinal Chem., Merck Res. Labs, Kenilworth, NJ

Abstract: BACE inhibitors are being developed for the treatment of Alzheimer's disease because of BACE1's obligate function in CNS A-beta peptide synthesis however the physiological functions of BACE1 and BACE2 proteases continue to emerge. We have observed that chronic treatment of mice with the BACE1 and BACE2 inhibitor, MBI-3, caused fur hypopigmentation after ~3 weeks of administration and manifested as patches of gray fur in black mice and light brown fur in agouti mice. Skin histology from MBI-3-treated mice identified reduced levels of melanin in the hair bulb and reduced melanin granules in the hair shafts with no evidence of pathology. Following drug washout, the hypopigmentation was completely reversible. In contrast to effects on fur, pigmentation of the eye (iris and retinal pigment epithelium) was unaffected. C57Bl/6 mice deficient for either Bace1 or Bace2 were not overtly hypopigmented and treatment with MBI-3 resulted in a hypopigmentation profile similar to that observed in MBI-3-treated wild type mice. This suggested that inhibition of either enzyme alone is not sufficient to cause hypopigmentation. mRNA expression profiling of anagen phase skin showed that MBI-3-mediated fur hypopigmentation was coincident with a dramatic and near complete suppression of multiple genes required for melanin synthesis (e.g., tyrosinase, TRP1). Treatment of mouse B16 melanoma cells with BACE inhibitors or siRNAs selective for BACE1 or BACE2 uncovered that the processing of the melanosome structural protein, PMEL17, was dependent on BACE2, but not BACE1. Further in vitro studies using purified BACE2 or BACE1

enzymes demonstrated BACE2 preferentially cleaved PMEL17-derived peptides and suggests that PMEL17 serves as a BACE2 substrate. However, since Bace2 knockout mice are not overtly hypopigmented, loss of BACE2 effects on PMEL17 processing does not sufficiently explain the effects of MBI-3 on fur pigmentation. In striking contrast to single gene targeted mice, Bace1/Bace2 double knockout C57Bl/6 mice displayed overt gray fur that was visually indistinguishable from the fur of MBI-3-treated mice. Furthermore, anagen phase skin from these mice expressed similar low levels of melanogenic genes that were not further reduced by MBI-3 treatment. These results suggest that BACE1 and BACE2 act to control transcriptional expression of melanogenic genes in hair bulb melanocytes and that BACE2 regulates PMEL17 processing. Whether the findings described here in mice will translate broadly to other species is unknown. This is, to our knowledge, the first example of an in vivo biological pathway that requires the activity of both BACE1 and BACE2 proteases.

Disclosures: **M.E. Kennedy:** A. Employment/Salary (full or part-time):; Merck & Co. **L.A. Hyde:** A. Employment/Salary (full or part-time):; Merck & Co. **L. Stahl:** A. Employment/Salary (full or part-time):; Merck & Co. **Y. Lin:** A. Employment/Salary (full or part-time):; Merck & Co. **T. Wisniewski:** A. Employment/Salary (full or part-time):; Merck. **M. Kavana:** A. Employment/Salary (full or part-time):; Merck & Co. **E.M. Parker:** A. Employment/Salary (full or part-time):; Merck & Co. **M. Smith:** A. Employment/Salary (full or part-time):; Merck & Co. **K. Tanis:** A. Employment/Salary (full or part-time):; Merck & Co. **R. Terry:** A. Employment/Salary (full or part-time):; Merck & Co. **L. Chen:** A. Employment/Salary (full or part-time):; Merck & Co. **D. Levitan:** A. Employment/Salary (full or part-time):; Merck & Co. **B. Weig:** A. Employment/Salary (full or part-time):; Merck & Co. **B. Cheewatrakoolpong:** A. Employment/Salary (full or part-time):; Merck & Co. **J. Cumming:** A. Employment/Salary (full or part-time):; Merck & Co. **J. Scott:** A. Employment/Salary (full or part-time):; Merck & Co. **K. Becker:** A. Employment/Salary (full or part-time):; Merck & Co. **S. White-Hunt:** A. Employment/Salary (full or part-time):; Merck & Co. **R.L. Peiffer:** A. Employment/Salary (full or part-time):; Merck & Co. **B.A. Mattson:** A. Employment/Salary (full or part-time):; Merck & Co.

Nanosymposium

672. Ischemia: Neuroprotection

Location: 152A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:00 AM

Presentation Number: 672.01

Topic: C.08. Ischemia

Title: Pyrrolidine dithiocarbamate extends the therapeutic window of tissue plasminogen activator in rat

Authors: *Z. ZUO;
Dept of Anesthesiol, Unvi of VA, CHARLOTTESVLE, VA

Abstract: Tissue plasminogen activator (tPA) is the only therapy approved by Food and Drug Administration for ischemic stroke. It has a narrow therapeutic window because its side effects will outweigh its benefits if it is used at a time point that is more than 3 h after the onset of brain ischemia, although this therapeutic window may be extended to 4.5 h in selective cases. The major side effects of tPA include hemorrhagic transformation (HT) and direct neurovascular toxicity. Due to this narrow therapeutic window, intravenous tPA is currently used only in < 4% patients with ischemic stroke. Pyrrolidine dithiocarbamate (PDTC) is an anti-oxidant and anti-inflammatory agent. We determine whether PDTC can extend the therapeutic window of tPA. When tPA was used 4 h after the onset of a thrombotic stroke in adult rats, there was an increase in hemorrhagic volumes in the brain and no improvement in infarct volume, brain edema and motor functions when compared to rats subjected to thrombotic stroke only. PDTC used at 30 min but not 4 h after the onset of stroke improved the neurological outcome. PDTC applied at 30 min after the onset of stroke blocked the increase of hemorrhagic volumes in the brain caused by tPA used at 4 h after the stroke. These results suggest that PDTC can be used to extend the therapeutic window of tPA.

Disclosures: Z. Zuo: None.

Nanosymposium

672. Ischemia: Neuroprotection

Location: 152A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:00 AM

Presentation Number: 672.02

Topic: C.08. Ischemia

Support: GAUK 604412

GACR P304/11/P386

GACR P304/12/G069

GACR P303/12/1464

TACR TE01020028

GACR P304/14/20613S

institutional support RVO: 67985823

Title: Anti-ischemic properties of NMDA receptor inhibitor and GABA-A receptor modulator, pregnanolone glutamate, in immature rats

Authors: *L. RAMBOUSEK[1,2,3], G. TSENOV[2], L. KLETECKOVA[3], K. VONDRAKOVA[2], K. VALES[2];
[1]Univ. of Zurich, Inst. of Pharmacol. and Toxicology, Zurich, Switzerland; [2]Inst. of Physiology, AS CR v.v.i., Prague, Czech Republic; [3]2nd Fac. of Medicine, Charles Univ., Prague, Czech Republic

Abstract: The perinatal hypoxic-ischemic insult frequently leads to mortality, morbidity and plays a key role in later pathological consequences. Ischemic insult causes a massive release of glutamate, consequent excitotoxic damage and neuroimmune response. There is therefore a need for safe and efficient therapeutic intervention.Pharmacological manipulation of NMDA and GABA-A receptors during early development can be challenging due to the switch from excitatory to inhibitory function of GABAergic neurons as well as the ongoing changes in NMDA receptor subunits composition (NR2B → NR2A). We propose that simultaneous allosteric modulation of NMDA and GABA-A receptors by neuroactive steroids derived from endogenous neruosteroids may represent a unique and safe therapeutic approach for the treatment of perinatal ischemia/hypoxia. In this study we investigated the effect of NMDA receptor inhibitor and GABA-A receptor modulator, 3α5β-pregnanolone glutamate (PG), in an animal model of focal cerebral ischemia in PD 12 rats. The focal cerebral ischemia was induced by the infusion of the endothelin-1 (ET-1, 40 pmol/ 1 μL) into the right dorsal hippocampus. We investigated the effect of PG on neurodegeneration and neuroinflammation changes induced by ET-1. The changes were assessed 1, 6, 13 and 26 days after the ischemic insult. To get a better mechanistic insight into the developmental and functional changes induced by ET-1, as well as the consequences of PG treatment, we investigated changes in the GABAergic and glutamatergic system using a battery of histological tests. We found strong neuroprotective effect of PG at doses 1 and 10 mg/kg (i.p., 5 min after the termination of ET-1 infusion). PG was capable to reduce neurodegeneration as well as the neuroinflammatory response. Furthermore PG protected parvalbumin positive GABA interneurons in the hippocampus. Taken together, PG represents a novel synthetic neuroprotective drug derived from endogenous neurosteroids that might be efficient in the treatment of perinatal brain insults such as ischemia or hypoxia.

Disclosures: L. Rambousek: None. **G. Tsenov:** None. **L. Kleteckova:** None. **K. Vales:** None. **K. Vondrakova:** None.

Nanosymposium

672. Ischemia: Neuroprotection

Location: 152A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:00 AM

Presentation Number: 672.03

Topic: C.08. Ischemia

Support: NIH R01NS064136

(AAHA grant in aid 10GRNT4200024

Title: NADPH oxidase is critical for the protective effects of ischemic postconditioning against stroke

Authors: *H. ZHAO[1], S. JOO[2], Y. FAN[2], W. XIE[2];
[1]Dept Neurosurg, Stanford Univ., STANFORD, CA; [2]Stanford Univ., Stanford, CA

Abstract: The underlying protective mechanisms of ischemic postconditioning (IPostC) remain elusive. Although previous studies have shown that IPostC generates protection by attenuating ROS activity, how this protection is achieved is not known. We hypothesize that inhibition of NADPH oxidase-mediated reactive oxygen species (ROS) is essential for the neuroprotective effects of IPostC. We used both wild type mice and mice mutant for the NADPH oxidase gene. Stroke was induced by middle cerebral artery (MCA) suture occlusion and IPostC was conducted by repeated, brief occlusions immediately after reperfusion. Glucose was injected to promote NADPH oxidase, while apocynin and 2-deoxyglucose (2-DG) were used to inhibit it. FACS was used to quantify inflammatory cells in the brain, and to assess ROS activity in macrophage/microglia subsets stained by 2′-7′-dichlorodihydrofluorescein diacetate (DCFH-DA). The results showed that IPostC reduced infarction as a function of the numbers of cycles of brief MCA occlusion. Glucose injection enlarged the infarction induced by the control stroke and abolished the protective effects of IPostC. Both 2-DG and apocynin injection reduced infarction, but only apocynin, and not 2-DG, acted synergistically with IPostC against stroke. Moreover, neither IPostC nor glucose altered the infarction in NADPH oxidase gene mutated mice. FACS results suggest that glucose injection exacerbated while IPostC inhibited inflammatory cell infiltration. Furthermore, IPostC blocked ROS activity in both macrophages and microglia. In conclusion, IPostC attenuated infarction by inhibiting NADPH oxidase-mediated ROS activity.

Disclosures: **H. Zhao:** None. **S. Joo:** None. **Y. Fan:** None. **W. Xie:** None.

Nanosymposium

672. Ischemia: Neuroprotection

Location: 152A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:00 AM

Presentation Number: 672.04

Topic: C.08. Ischemia

Support: NIH HL-065380

NIH HL-077731

NIH HL-093554

Title: Ischemic post-conditioning by targeting mitochondria improved outcome of stroke in rat

Authors: *I. RUTKAI, S. DUTTA, P. V. G. KATAKAM, D. W. BUSIJA;
Pharmacol., Tulane Univ., New Orleans, LA

Abstract: Growing evidence suggests that mitochondria play an important role in the pathophysiology of stroke. Currently, the limited-time thrombolytic therapy is the only accepted treatment for occlusive stroke, but studies in our laboratory have shown that pre-conditioning by targeting mitochondria has a beneficial role following stroke under in vivo and in vitro conditions. Recently, we found preserved mitochondrial derived vasoreactivity in middle cerebral arteries (MCAs) from the side ipsilaterial (IPSI) to experimental stroke despite diminished vascular responses of IPSI MCAs to acetylcholine (Ach), bradykinin (BK), and sodium nitroprusside (SNP) compared with contralateral (CONTRA) and control MCAs. Post-conditioning may provide similar benefits as pre-conditioning and be more efficacious for stroke since its occurrence is unpredictable. We tested whether pharmacologically induced post-conditioning by targeting mitochondria using diazoxide (DZ), one of the selective mitochondrial ATP-sensitive potassium (mitoKATP) channel activators, improved stroke outcome focusing on endothelium related vascular functions. Male Sprague Dawley rats were subjected to 90 min ischemia by MCA occlusion and 48 h reperfusion. Rats were subjected to intraperitoneal injections of 10mg/kg DZ at 60 min post ischemia and after 24 h reperfusion. The IPSI and CONTRA MCAs were isolated and vascular function was investigated using videomicroscopy. The brain was sliced and the infarct volume was defined using 2,3,5-triphenyltetrazolium chloride (TTC) staining. DZ treatment significantly decreased the infarct volume from 60.3±2.7% to 32.5±4.8% (expressed as percentage of IPSI hemisphere). The IPSI MCAs of DZ treated rats showed significantly improved vasodilation in response to 10 μM Ach (from 3.03±1.88 μm to 30.16±11.08 μm), 10 μM BK (from 27.21±3.66 μm to 58.18±3.86 μm), and 10 μM SNP (from 37.35±3.17 μm to 79.98±14.42 μm). Post-conditioning further improved

mitochondrial derived vasoreactivity in IPSI MCAs to DZ (50 μM: 22.62±3.37 μm to 55.88±15.76 μm and 100 μM: 38.64±4.95 μm to 67.05±18.20 μm; respectively). Our results suggest that targeting mitochondria after ischemia reperfusion injury reduces infarct volume and improves vascular function especially in endothelium, contributing to a better outcome and recovery from stroke when compared with the non-treated group.

Disclosures: I. Rutkai: None. **S. Dutta:** None. **P.V.G. Katakam:** None. **D.W. Busija:** None.

Nanosymposium

672. Ischemia: Neuroprotection

Location: 152A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:00 AM

Presentation Number: 672.05

Topic: C.08. Ischemia

Title: Functional neuroprotection for the ischemic neonatal brain with lps preconditioning

Authors: *R. ASKALAN[1], M. A. PERRY[2], A. TASKER[2];
[1]Hosp. For Sick Children Res. Inst., Toronto, ON, Canada; [2]Univ. of Prince Edward Island, Charlottetown, PE, Canada

Abstract: Background: We have shown that pre-treatment of seven-day old (P7) rat pups (which correspond in brain development to human term newborn) with a low dose of lipopolysaccharide (LPS) will result in 90% reduction in ischemia-induced brain damage. However, LPS -induced preconditioning will only be a valuable therapy for babies at high risk of ischemic brain injury if the reduction in tissue damage translates into meaningful functional improvement. We sought to determine if preconditioning with LPS prior to hypoxic-ischemic (HI) injury confers long-term neuroprotection. Methods: P7 rats were randomly assigned to LPS or saline treated group. The LPS treated group received an intraperitoneal injection of 0.1mg/kg LPS. Forty eight hours after LPS or normal saline injection, the right internal carotid artery was occluded and then subjecting the animal to hypoxia (8% oxygen for 65min resulting in ischemic injury in the hemisphere ipsilateral to the occlusion. Functional outcome was evaluated over 90 days using a comprehensive and validated battery of behavioral tests for physical development (incisor eruption, auditory startle, eye opening), sensory and neuromotor function (olfactory orientation, forelimb grip strength, wire mesh ascend, open field activity), emotionality (open field and elevated plus maze) and cognition/attention (spontaneous alteration, radial arm and water maze, prepulse inhibition). Results: LPS pre-treated rats had consistently greater forelimb grip strength than saline/HI rats over the entire time course measured (P11-P23) and relied less on the use of

hind limbs to maintain grip. Similarly, LPS preconditioned rats were consistently faster at ascending an inclined wire mesh to reach a littermate each day from P12-P17. We also found that rats preconditioned with LPS were significantly more active in the open field on P20 than saline preconditioned animals. Differences were trending toward significance were found in olfactory orientation (p=0.65) and other behaviors in the open field on P20. In post-weaning analyses of open field behavior (P34 and P75) and performance in the Morris water maze (P83-86), we were unable to detect significant differences between conditions with the exception of swim speed (LPS/HI significantly faster than SAL/HI). Conclusion: Collectively these data support the premise that LPS preconditioning has the potential to confer long-term functional neuroprotection. These findings are the first step in bringing this powerful neuroprotective phenomenon to the bedside of babies who have a predictable high risk of ischemic brain injury (e.g. children with congenital heart disease) and change their outcome.

Disclosures: R. Askalan: None. **A. Tasker:** None. **M.A. Perry:** None.

Nanosymposium

672. Ischemia: Neuroprotection

Location: 152A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:00 AM

Presentation Number: 672.06

Topic: C.08. Ischemia

Support: NIH Grant HL082485, HL86576

Veterans Affairs Merit Award NURC-051010F

Title: Targeted complement inhibition reduces cerebral ischemia-reperfusion injury and improves functional outcome in sub-acute phase of murine ischemic stroke

Authors: *A. M. ALAWIEH[1], A. ELVINGTON[2], H. ZHU[2], J. YU[1], C. ATKINSON[2], M. KINDY[1], S. TOMLINSON[2];
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Abstract: Cerebral ischemia-reperfusion injury is associated with a cascade of inflammatory events, and complement activation has been shown to play an important role in inflammation and secondary injury after ischemic stroke. Nevertheless, complement has many important physiological functions, and complement activation products have been implicated in repair and regenerative mechanisms within the CNS, including following ischemic stroke. We have

previously shown that injury site targeted complement inhibitors CR2-Crry (inhibits all complement pathways at C3 activation) and CR2-fH (inhibits only the alternative pathway) are both protective in an acute murine model of ischemic stroke. Here we investigated the effects of these inhibitors, as well as C3 deficiency, on injury and recovery in the subacute phase of ischemic stroke. Adult male C3 deficient or wild type C57BL/6 mice were subjected to transient middle cerebral artery occlusion (MCAO) for 60 minutes. Wild type mice were treated with a single dose of CR2-Crry, CR2-fH or vehicle control by tail vein injection 30 minutes post-reperfusion. Histological, functional and cognitive outcomes were assessed at 1, 3 and 7 days after MCAO (n=12-18/ group). C3 deficiency or treatment of wild type mice with either CR2-Crry or CR2-fH significantly reduced infarct volume and inflammatory markers at 24 hours and 7 days following ischemic stroke, but only CR2-fH treated mice had no increase in infarct volume between 24 hours and 7 days. Furthermore, whereas C3 deficiency and both inhibitors improved neurological deficit at 3 days post-ischemia, at 7 days only CR2-fH treated mice had improved deficit scores and increased locomotor activity. CR2-fH treated mice also showed improved performance on spatial learning and passive avoidance tasks compared to vehicle treated mice. The improvements in outcome of CR2-fH treatment vs. CR2-Crry treatment were associated with increased subventricular zone neurogenesis and increased relative VEGF expression. In addition, C3 deficiency, but not inhibitor treatment, resulted in increased mortality compared to controls, an outcome linked to an increased susceptibility to infection in C3 deficient mice. In conclusion, transient and targeted inhibition of complement reduces acute inflammation and injury after stroke. However, compared to inhibition of all activation pathways, specific inhibition of the alternative pathway provided additional improvements in outcome, which may be due to quantitative differences in the level of complement activation. Finally, targeted complement inhibition did not increase susceptibility to secondary complications after experimental stroke.

Disclosures: **A.M. Alawieh:** None. **A. Elvington:** None. **H. Zhu:** None. **J. Yu:** None. **C. Atkinson:** None. **M. Kindy:** None. **S. Tomlinson:** None.

Nanosymposium

672. Ischemia: Neuroprotection

Location: 152A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:00 AM

Presentation Number: 672.07

Topic: C.08. Ischemia

Support: AHA Grant 10SDG3540003 For Jennifer Lee-Summers

Title: Rewarming from therapeutic hypothermia induces apoptosis of cortical neurons in a swine model of neonatal hypoxic-ischemic encephalopathy

Authors: *B. WANG, J.-H. LEE, J. ARMSTRONG, M. REYES, E. KULIKOWICZ, D. SPICER, U. BHALALA, Z. YANG, R. KOEHLER, M. LEE, J. LEE-SUMMERS;
ACCM, Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Severe neurologic disabilities persist in survivors of neonatal hypoxic-ischemic encephalopathy (HIE) despite therapeutic hypothermia. Rewarming could reduce neuroprotection from hypothermia. We investigated whether rewarming induces neuronal apoptosis in the cerebral cortex of a swine model of HIE. Neonatal piglets (3-5 days old) received sham surgery or hypoxic-ischemic injury (HI) via hypoxic-asphyxic cardiac arrest and resuscitation with recovery under 1) normothermia; 2) overnight hypothermia; 3) hypothermia + rapid rewarming (4°C/h); or 4) hypothermia + slow rewarming (0.5°C/h). Some piglets received arrest + hypothermia + rapid rewarming with subdural administration of a non-peptide caspase-3 inhibitor or artificial cerebral spinal fluid (aCSF). Naïve piglets that did not receive anesthesia or surgery were prepared as an additional control group. Piglets were euthanized at 29 h after resuscitation or sham surgery and the brains were prepared for histological and biochemical measurements. Apoptotic profiles observed by hematoxylin and eosin staining and TUNEL+ cells were quantified in motor and piriform cortex, and cleaved caspase-3 levels in sensorimotor and piriform cortex were assessed by western blotting. Naïve (n=7) and sham-operated (n=8) piglets had similar numbers of apoptotic and TUNEL+ cells ($p \geq 0.40$). HI injured, rapidly rewarmed piglets (n=8) had more apoptotic profiles in motor cortex than piglets that remained hypothermic (n=8; $p<0.05$) or normothermic (n=6; $p<0.05$) after HI and naïve/sham piglets (n=15; $p<0.05$). Piglets that were slowly rewarmed after HI (n=8) had more apoptosis in motor cortex than piglets that remained hypothermic after HI ($p<0.05$) and naïve/sham piglets ($p<0.05$). In comparison to those that remained hypothermic (n=7), both slowly (n=8; $p<0.05$) and rapidly rewarmed (n=7; $p<0.05$) HI injured piglets had more TUNEL+ cells in motor cortex. Necrotic profiles were observed in HI injured, normothermic piglets. HI injured piglets that were rapidly rewarmed (n=4) had more cleaved caspase-3 in sensorimotor and piriform cortex than those that were slowly rewarmed (n=4; $p<0.05$) or that remained hypothermic (n=4; $p<0.05$). In piglets that received HI with rapid rewarming, subdural administration of the caspase-3 inhibitor (n=6) reduced the number of apoptotic and TUNEL+ profiles in motor and piriform cortex in comparison to those that received aCSF (n=6; $p<0.05$). We conclude that therapeutic hypothermia with rewarming after HI injury causes caspase-dependent apoptosis of neurons in cerebral cortex that can be managed pharmacologically with a caspase inhibitor.

Disclosures: B. Wang: None. **J. Lee:** None. **J. Armstrong:** None. **M. Reyes:** None. **E. Kulikowicz:** None. **D. Spicer:** None. **U. Bhalala:** None. **Z. Yang:** None. **R. Koehler:** None. **M. Lee:** None. **J. Lee-Summers:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Jennifer Lee-Summers has received research funding from Covidien in the past..

Nanosymposium

672. Ischemia: Neuroprotection

Location: 152A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:00 AM

Presentation Number: 672.08

Topic: C.08. Ischemia

Support: SHC Grant 87100-PHI-14 (TSF)

SHC Grant 85120-PHI-14 (TSF)

Moulder Center for Drug Discovery

SHC Grant 85110-PHI-14 (SHR)

NIH Grant NS086570-01 (SHR)

Title: Therapeutic interventions in a mouse model of cerebral palsy

Authors: *D. FEATHER-SCHUSSLER[1], N. MCCORMACK[1], H. DYKSTRA[2], W. CHILDERS[4], B. BLASS[4], M. ABOU-GHARBIA[4], S. RAWLS[3], S. H. RAMIREZ[2], T. S. FERGUSON[5];
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Abstract: Cerebral palsy (CP) is a non-progressive motor disorder affecting 1:300 live births annually. However, the exact cause of CP is unknown. Periventricular leukomalacia (PVL), or white matter damage, is a hallmark of CP. Hypoxia and ischemia are classic injuries causing excitotoxicity, leading to PVL and neuron damage in humans. Recently, infection and inflammation have been implicated in CP. Our model uses a post-natal day 6 mouse pup. CP is induced by the combination of Hypoxia, Ischemia, and inflammation (lipopolysaccharide, LPS) (HIL), which better mimics PVL seen in humans. With this model, we study the underlying cellular causes of CP, as well as a potential diagnostic tool and treatments. In our CP model, we find elevated S100β levels, a biomarker indicating astrocyte cell death, in HIL blood samples. In addition, we note significant astrocyte loss 12-18hrs after injury, suggesting that S100beta may be a potential diagnostic biomarker of neonatal brain injury. Neuronal and white matter loss does not occur until 48hrs after injury and then progresses up to 1wk post-injury. Early astrocyte loss may lead to neuron and oligodendrocyte cell loss. As a result, there may be a treatment window for CP between 0-48hrs after injury, before significant oligodendrocyte and neuronal cell loss

occurs. Our goal is to prevent CP using drug treatment in neonatal mice. Astrocytes are responsible for removing glutamate from the synapse of neurons, primarily through the glutamate transporter GLT-1. We are testing three drugs that increase GLT-1 protein expression: clavulanic acid (CA) and two compounds developed by the Moulder Center for Drug Discovery at Temple University, 093 and 031. GLT-1 protein expression is low immediately after injury. All three compounds (CA, 093, and 031) increase GLT-1, even when administered after injury. Short-term visual memory deficits, demonstrated by a novel object recognition test, as well as neonatal and adult motor deficits, determined by a battery of motor tests, are also improved following drug treatment. Once CP is established, surgery and physical therapy, as well as pharmacological intervention address the motor deficits seen in patients. However, a significant number of patients with CP have cognitive disabilities that are not addressed with the current standard of care. Our goal was to introduce exercise as a potential therapy for both the motor and cognitive deficit. Exercise is beneficial for many diseases and disorders, including neurological disorders. By placing adult HIL mice in a 3 week exercise program, we demonstrate improvement in not only their motor deficits, but also in their cognitive loss.

Disclosures: **D. Feather-Schussler:** None. **N. McCormack:** None. **H. Dykstra:** None. **W. Childers:** None. **B. Blass:** None. **M. Abou-Gharbia:** None. **S. Rawls:** None. **S.H. Ramirez:** None. **T.S. Ferguson:** None.

Nanosymposium

672. Ischemia: Neuroprotection

Location: 152A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:00 AM

Presentation Number: 672.09

Topic: C.08. Ischemia

Support: AHA grant 11GRNT7370069

Title: Estrogen receptor beta regulates inflammasome activation in the hippocampus of female rats

Authors: ***A. P. RAVAL**[1], H. H. PATEL[1], F. J. BRAND III[2], H. BRAMLETT[2], J. DE RIVERO VACCARI[2];
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Abstract: Although chronic 17β-estradiol (E_2) has been shown to be a neuroprotective agent in animal brain injury models, concern regarding its safety was raised by the failed translation of

this phenomenon to the clinic. In contrast with chronic E_2 treatment, our recent study demonstrated that periodic treatment with an estrogen receptor subtype beta (ER-β) agonist reduces post-ischemic hippocampal injury in young ovariectomized rats. Since the majority of ischemic events in women occur after menopause onset, it is crucial that we confirm the efficacy of ER-β agonist treatment in reproductively senescent females. Furthermore, inflammatory molecules produced during menopause can stimulate an innate immune responses in the brain and can exacerbate ischemic damage. A key component of the innate immune response is the inflammasome. The inflammasome is a multiprotein complex responsible for activation of caspase-1 and processing of the inflammatory cytokines IL-1β and IL-18. The inflammasome is comprised of caspase-1 (C1), the caspase recruitment domain (ASC), and a pattern recognition receptor such as a NOD-like receptor (NLR). In the current study, we tested the hypothesis that periodic ER-β agonist treatment reduces inflammasome activation in the hippocampus of reproductively senescent female rats. We tested the proposed hypothesis using retired breeder (9-11 months) Sprague-Dawley female rats. The estrous cycle was monitored by examining vaginal smears daily and was confirmed by virtually undetectable plasma levels of E_2. Rats in constant diestrus were considered reproductively senescent. Rats were exposed to periodic ER-β agonist (DPN; 1 mg/kg; vehicle DMSO) treatment at 48 or 72 or 96 hour intervals for 21 days. Rats were sacrificed at 48 or 72 or 96 hours after last treatment. We collected hippocampal tissue and performed Western blotting for C1, ASC and IL-1β to determine the effect of senescence. The results demonstrated a significant decrease of the inflammasome proteins caspase-1 ($p<0.002$), ASC ($p<0.03$) and IL-1β ($p<0.02$) in the hippocampus of ER-β agonist treated rats. Silencing of hippocampal ER-β attenuated E_2-mediated decrease in inflammasome proteins, suggesting a role of ER-β in regulation of inflammasome activation. This study emphasizes the need to investigate a periodic ER-β agonist regimen to reduce the innate immune response in the brain, and cerebral ischemia incidence/impact in post-menopausal women.

Disclosures: A.P. Raval: None. **H.H. Patel:** None. **F.J. Brand III:** None. **H. Bramlett:** None. **J. de Rivero Vaccari:** None.

Nanosymposium

672. Ischemia: Neuroprotection

Location: 152A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:00 AM

Presentation Number: 672.10

Topic: C.08. Ischemia

Support: NS34179 (CI)

NS67078 (PZ)

DFG Fellowship (AK)

Title: Neuronal overexpression of the mitochondrial protein prohibitin1 is markedly neuroprotective after focal cerebral ischemia

Authors: *A. KAHL, C. ANDERSON, G. MANFREDI, C. IADECOLA, P. ZHOU;
Feil Family Brain and Mind Res. Inst., Weill Cornell Med. Sch., New York City, NY

Abstract: Prohibitin (PHB) is a ubiquitous protein essential for the maintenance of mitochondrial structure and function (Genes Dev 22:476, 2008). In vitro, overexpression of PHB is cytoprotective in different neuronal injury modalities, an effect associated with a suppression of complex I-dependent generation of reactive oxygen species (ROS) (J. Neurosci 32:583, 2012). In a mouse model of transient forebrain ischemia, viral-mediated PHB gene transfer protects hippocampal CA1 neurons by reducing postischemic ROS formation and cytochrome C release (Stroke 45:1131, 2014). However, it is not known whether PHB expression specifically in neurons is sufficient to increase the resistance of the brain to ischemia, and whether PHB expression reduces brain injury also in focal cerebral ischemia, the most common type of stroke in humans. In order to address these questions we generated transgenic mice, in which PHB overexpression is driven in forebrain neurons by the CaMKIIα promoter. CaMK-PHB mice are viable, develop normally and exhibit normal cage behavior. At 2-3 months of age, no differences in systolic arterial pressure (SAP) and body weight (BW) were observed between CaMK-PHB mice (SAP: 127±8.1mmHg; BW: 21.3±0.4g; mean±SE) and wild type (WT) littermates (SAP: 114±2.9mmHg; BW: 21.0±0.9g; $p>0.05$). As predicted by the spatial expression pattern of the CaMKIIα promoter, PHB protein was increased in neocortex (51±7%; n=3) and hippocampus (62±8%), but not in striatum, cerebellum, or in peripheral organs. We subjected CaMK-PHB mice to focal cerebral ischemia by transient occlusion of the middle cerebral artery (MCAO). MCAO produced comparable reductions in cerebral blood flow in the ischemic territory (WT: 91±2%; CaMK-PHB: 91±2%; $p>0.05$). However, the resulting infarct volume, assessed 72 hrs later in Nissl stained sections, was significantly smaller in CaMK-PHB mice (12.0±1.5 mm3) than in WT mice (52.1±9 mm3; $p<0.029$; n=4/group). The reduction in tissue damage in CaMK-PHB mice correlated with an increased latency to fall at the hanging wire test (WT: 32±14s; CaMK-PHB 51±9s; $p<0.05$) and improved sensory-motor performance at the corner test (% of turns to ipsilateral side: CaMK-PHB: 41±5 %; WT: 92±3%; $p<0.05$). We conclude that increased neuronal expression of PHB is sufficient to confer a remarkable protective effect in focal cerebral ischemia, associated with a substantial functional improvement. The mechanisms of the protection remain unclear, but they may involve increased mitochondrial integrity and suppression of ROS production. Modulating PHB expression could serve as a new preventive or therapeutic strategy to protect the brain from ischemic brain injury.

Disclosures: **A. Kahl:** None. **C. Anderson:** None. **G. Manfredi:** None. **C. Iadecola:** None. **P. Zhou:** None.

Nanosymposium

672. Ischemia: Neuroprotection

Location: 152A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:00 AM

Presentation Number: 672.11

Topic: C.08. Ischemia

Support: CIHR-POP 20R06558

Title: Protease-activated anti-inflammation therapy for ischemic stroke involves NF kappa B and Caspase 8 signaling pathways in the penumbra area

Authors: ***S. ZHANG**[1,2], L. KOJIC[2], Y. WEN[2], D. QIANG[2], F. MORIN[2], M. S. CYNADER[2], W. JIA[1,2];
[1]Dept. of Surgery, Fac. of Medicine, Univ. of British Columbia, [2]Brain Res. Ctr., Vancouver, BC, Canada

Abstract: Regulation of inflammation in the acute stages of stroke is crucial to achieve neuroprotection. We have genetically engineered chimeric transmembrane proteins that express anti-inflammatory factors. In addition, the anti-inflammatory fragment of the chimeric protein is subject to cleavage and release by brain proteases that are activated during ischemic stroke. Prophylactic treatment with these chimeric constructs delivered in the rat MCAO model using AAV vectors results in lasting and robust neuroprotection. We found that our construct was expressed in both neuronal and microglial cells. Expression of activated Caspase 8 and NF kappa B in the penumbra area of the ischemic striatum and cortex were significantly reduced in the prophylactically treated (and protected) animals. Levels of activated Caspase 8 were negatively correlated with the expression level of the chimeric protein. These results indicate that our strategy of prophylactic delivery of an anti-inflammatory construct that is released by stroke-activated proteases is a feasible approach. Down-regulation of the NF kappa B related signaling pathway appears to at least partly responsible for the neuroprotection observed. Detailed studies are ongoing to examine the effects of this strategy in different cell populations of the penumbra area.

Disclosures: **S. Zhang:** None. **L. Kojic:** None. **Y. Wen:** None. **D. Qiang:** None. **F. Morin:** None. **M.S. Cynader:** None. **W. Jia:** None.

Nanosymposium

672. Ischemia: Neuroprotection

Location: 152A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:00 AM

Presentation Number: 672.12

Topic: C.08. Ischemia

Title: RAGE activation-mediated neuroinflammation aggravates subarachnoid hemorrhage (SAH)-associated pial arteriolar dilating dysfunction and neuropathy in rats

Authors: *H.-L. XU[1], C. PAISANSATHAN[1], Y. WU[1], F. TESTAI[2], D. PELLIGRINO[1]; [1]Neuroanesthesia Res. Lab., [2]Dept. of Neurol., Univ. Illinois Chicago, CHICAGO, IL

Abstract: Cerebral microvasculature dysfunction plays an important role in mediating SAH-associated brain injury. Although the underlying mechanism is not completely understood, accumulating evidence indicates that post-SAH neuroinflammation is critical to its occurrence. Our recent findings indicated that, in SAH, the expression of the receptor for advanced glycation end-products (RAGE) is elevated. In this study, we tested the following hypotheses: (1) SAH-associated neuroinflammation is mediated through a RAGE activation-related pathway; (2) blocking RAGE activation, using a selective RAGE inhibitor, FPS ZM-1, attenuates SAH-induced neuroinflammation, and restores cerebrovascular dilating function; and (3) inhibiting RAGE activating pathway improves SAH-related neurological impairment. Rats were randomized into three groups: sham surgical controls, vehicle-treated SAH controls, and FPS ZM-1-treated SAH rats. The SAH model involved endovascular perforation of the anterior cerebral artery. Neuroinflammation [was represented by pial venular leukocyte adhesion (PVLA)] and cerebrovascular dilating function (represented by pial arteriolar responses to topically-applied acetylcholine) were evaluated via an intravital microscope mounted over a closed cranial window. PVLA was expressed as the % pial venular area occupied by adherent rhodamine-6G-labeled leukocytes. Neurobehavioral function, which included spontaneous activity, muscle tone, and neurologic reflex, was evaluated during the post-SAH recovery period. Compared to the sham surgical group, a marked increased PVLA was observed in vehicle-treated animals (10.28±1.70 % vs 3.67±0.24 % in sham), with a profound leukocyte extravascular infiltration revealed during an extended observation period. No sign of infiltration was found in the sham surgical group. Treatment with FPS ZM-1 at 1 mg/kg and 5 mg/kg dose-dependently decreased SAH-associated PVLA to 7.76± 0.79% and 3.31± 0.58 % respectively, with complete suppression of leukocyte extravascular migration. SAH led to markedly attenuation of acetylcholine-elicited pial arteriolar dilation, which can be restored completely by the presence of FPS ZM-1. Additionally, a significant improvement in SAH-related neurological deficits was

also found in FPS ZM-1-treated animals. In summary, RAGE activation plays an important role in mediating post-SAH neuroinflammation, which may contribute to SAH-associated cerebrovascular dilating dysfunction and neurological impairment.

Disclosures: H. Xu: None. **C. Paisansathan:** None. **Y. Wu:** None. **F. Testai:** None. **D. Pelligrino:** None.

Nanosymposium

673. Metabolic and Excitotoxic Mechanisms of Cell Death and Degeneration

Location: 144A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:30 AM

Presentation Number: 673.01

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH NEI 2R01EY012345

Title: The effect of CD3 in excitotoxic dendritic degeneration of the mouse retina

Authors: *K. HUANG, P. WANG, N. TIAN;
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Abstract: One of the major causes for neuronal cell death following insults such as traumatic brain injury, spinal cord injury, or traumatic optic neuropathy is glutamate excitotoxicity. The initial insult damages neurons, which leads to an excessive amount of glutamate released from injured neurons and calcium influx on the surrounding neurons that ultimately induces additional neuronal death. In the retina, it has been shown that there is a significant loss or re-organization of the retinal ganglion cell (RGC) dendrites prior to cell death. This results in a loss of synaptic inputs and is thought to play an important role for the subsequent apoptotic processes and cell death. We hypothesize that CD3zeta, a protein normally associated with the immune system, plays an integral role in retinal ganglion cell dendritic collapse in response to NMDA mediated excitotoxicity. We show through time lapse imaging that different retinal ganglion cell subtypes have different responses to NMDA perfusion on explanted retinas. Alpha type RGCs exhibit rapid and total dendritic collapse one hour after NMDA exposure, while JamB and BD cells retain up to 80% of their dendrites five hours after NMDA perfusion. This implies that JamB and BD RGCs are naturally more resistant to the effects of NMDA mediated toxicity. In CD3zeta deficient mice, NMDA perfusion results in complete dendritic collapse of JamB and BD RGCs at one hour after exposure, mirroring the vulnerability of alpha type RGCs to NMDA. These

results provide evidence that CD3 may function in mechanisms that lead to dendritic collapse upstream of excitotoxic cell death.

Disclosures: **K. Huang:** None. **N. Tian:** None. **P. Wang:** None.

Nanosymposium

673. Metabolic and Excitotoxic Mechanisms of Cell Death and Degeneration

Location: 144A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:30 AM

Presentation Number: 673.02

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Ente Cassa di Risparmio di Firenze

University of Florence

Title: PARP-1 activation increases Ca2+-permeable AMPA receptors in the CA1 region of hippocampal pyramidal cells and causes selective and delayed neuronal death

Authors: ***F. MORONI**[1], E. GERACE[2], A. MASI[2], F. RESTA[2], R. FELICI[2], E. LANDUCCI[2], T. MELLO[2], D. E. PELLEGRINI-GIAMPIETRO[2], G. MANNAIONI[2];
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Abstract: Alkylating DNA agents such as N-methyl-N'-nitro-N'-nitrosoguanidine (MNNG) are known to trigger a form of programmed cell death that may be helpful in understanding the mechanisms that may occur in post-ischemic brain damage. It has been previously proposed that MNNG and PARP activation induces a newly described cell death that is characterized by mitochondrial release and nuclear translocation of apoptosis-inducing factor (AIF). To further investigate the mode of cell death induced by MNNG, we set up an in vitro model by exposing organotypic hippocampal slices to different concentrations of MNNG for various periods of time. MNNG induced a dose-dependent neurodegeneration in hippocampal slices, and when used at 100 μM for 5 min it caused a rather selective and delayed (48 h later) degeneration of the pyramidal cells in the CA1 hippocampal layer. Exposure to MNNG was associated with a strong increase in the activity of poly(ADP-ribose) polymerase (PARP) and the notable consumption of NAD+ and ATP. MNNG did not increase caspase-3 activity, evaluated either with fluorescence approaches or by monitoring PARP-1 cleavage, suggesting that the caspase pathway is not involved in this type of cell death process. Furthermore, experiments performed using either immunohistochemistry or standard accurate separation of the mitochondria or nuclei fractions

failed to demonstrate translocation of AIF or cytochrome-C. Electrophysiological experiments showed that PARP activation was associated with a significant modulation of the neuronal membrane properties. Hence, we firstly investigated the suggested ability of ADP-ribose to open TRPM2 channels in MNNG-induced cell death: we found that TRPM2 were not involved in this model of neuronal death. We then observed, using biochemical and electrophysiological approaches, increased levels of GluA1 and a relative decrease of GluA2 subunits and an I-V curve rectification of the AMPA channels in the CA1 but not in the CA3 region. The AMPA receptor antagonist NBQX and the selective Ca2+ permeable AMPA channel blocker NASPM reduced MNNG-induced CA1 pyramidal cell death. The NMDA receptor antagonist MK-801 or the mGlu1 receptor antagonist 3-MATIDA had no protective actions. Our results show that activation of the nuclear enzyme PARP-1 modulates the expression of membrane proteins and Ca2+ permeability of AMPA channels, thus affecting the function and survival of CA1 pyramidal neurons.

Disclosures: **F. Moroni:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Owner of PARP inhibitor patent. **E. Gerace:** None. **A. Masi:** None. **F. Resta:** None. **R. Felici:** None. **E. Landucci:** None. **T. Mello:** None. **D.E. Pellegrini-Giampietro:** None. **G. Mannaioni:** None.

Nanosymposium

673. Metabolic and Excitotoxic Mechanisms of Cell Death and Degeneration

Location: 144A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:30 AM

Presentation Number: 673.03

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: HKUST start-up funding (R9321)

National Key Basic Research Program of China (2013CB530900)

National Institute of Health (NS71022)

Title: Essential roles of NF-κB in cytokine mediated neuroprotection in normal and ATM-deficient neurons

Authors: ***T. HUI**[1], K. HERRUP[1,2];
[1]Life Sci., Hong Kong Univ. of Sci. and Technol., Hong Kong, Hong Kong; [2]Dept. of Cell Biol. and Neurosci., Rutgers Univ., Piscataway, NJ

Abstract: Nuclear factor-κB (NF-κB) is well known for its roles in the inflammatory response to injury and the immune response to foreign invaders. In central nervous system, NF-κB proteins are widely expressed in neurons and glial cells where they participate in the control of cell growth and survival. They are also help mediate inflammation-induced alterations in synaptic plasticity, behavior and cognition. We report here that TNFα and IL1β each activates different NF-κB proteins in differentiated N2a cells (a neuroblastoma cell line) as well as in cultured primary neurons. TNFα reduced, while IL1β stimulated, the NF-κB pathway including the downstream activation of death-promoting MAP kinase signals. TNFα suppressed the induced neuronal cell cycle reentry while IL1β elevated this neurotoxic response. Further, the NF-κB proteins and MAP kinases responded differently to these two different pro-inflammatory cytokines. This differential cytokine response is also apparent in vivo. Wild type (WT) and ATM-deficient Atmtm1Awb (Awb) mice were injected i.p. with TNFα, IL1β or with LPS - a global inflammatory stimulus - for 4 successive days. Our findings echoed those of our in vitro systems. TNFα suppressed DNA damage and cell cycle re-entry in Awb Purkinje neurons while IL1β and LPS induced the opposite reaction. Similarly, TNFα reduced the death-promoting MAP kinases in wild type cortex, while LPS acted in the opposite manner. We present data suggesting that the neuroprotective effects of TNFα might be related to the nuclear localization of p50 NF-κB subunit as well as to A20 expression. Finally, we found that the NF-κB response was different in Awb animals. LPS significantly increased both the nuclear localization and expression levels of the p50 NF-κB subunit in cortex and cerebellum, thus reducing expression of death-promoting MAP kinases. Our data take on increase relevance as they may explain why neurons with accumulated DNA damage in ATM-deficient (Awb) mice remain viable. Taken together, our study suggests that TNFα, by selective activation of the NF-κB pathway is neuroprotective.

Disclosures: **T. Hui:** None. **K. Herrup:** None.

Nanosymposium

673. Metabolic and Excitotoxic Mechanisms of Cell Death and Degeneration

Location: 144A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:30 AM

Presentation Number: 673.04

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Science Foundation of Ireland (08/IN1/1949)

Title: Differential impact of physiological and excitotoxicity-induced NF-kB activation on ankyrinG expression

Authors: *H.-G. KOENIG, R. F. SCHWAMBORN, J. H. M. PREHN;
Physiol. & Med. Physics, Royal Col. of Surgeons In Ireland, Dublin, Ireland

Abstract: The transcription factor NF-κB exhibits constitutive and injury-induced activation in neurons and is crucial for the development of the nervous system. The cytoskeletal scaffold protein AnkyrinG accumulates at the axon-initial segment (AIS) and is regarded as the prime organizer for subsequent attachment of AIS-localized ion channels. Neuronal activity-dependent proximal-to-distal relocation of the AnkyrinG extension profile along the AIS was formerly shown to fine-tune neuronal excitability in a homeostatic manner, while the protein is degraded following ischemic events. We previously provided evidence for a constitutively-active NF-κB cascade in the axon-initial segment, which co-localized to ankyrinG. IKKα/β, NEMO and p65 all associated with ankyrinG at the AIS following detergent-extraction, and IKK interaction with the scaffold was evidenced in immunoprecipitation and proximity-ligation assays. We here report that ank3 (ankyrinG) transcripts were potently up-regulated by p65 activation, this however depended on Ser536 phosphorylation status. Transfection of plasmids encoding a Ser536Ala-p65 mutant protein expression showed a more pronounced ank3-transcriptional induction than phospho-mimetic p65. Phosphorylated IKKα/β levels at the AIS were decreased following excitotoxic, extra-synaptic NMDA application that also resulted in reduced ankyrinG immunofluorescence at the AIS. RNAi-induced depletion of ankyrinG in cortical neurons resulted in ablation of phosphorylated IKKα/β in immunofluorescence analyses. Notably, ankyrinG depletion enhanced (2000 kB-5') ank3-promoter dependent luciferase reporter activity in a p65-dependent manner. In turn, ankyrinG overexpression facilitated phosphorylation of the IKKα/β activation-loop, as well as phosphorylation of the transactivation-domain site (Ser536) on p65/NF-κB in Western-blot and immunofluorescence analyses. Moderate KCl-induced depolarisation or synaptic NMDA-receptor activation increased p65 phosphorylation and trans-activation of a Ser536-p65 phosphorylation-dependent κB-response element reporter in cortical neurons, while these stimuli all diminished ank3-promoter-dependent reporter expression. We thus provide data suggesting that the NF-κB cascade serves in a feedback-loop controlling AnkyrinG expression depending on p65 phosphorylation status, which depends on the nature of the excitatory stimulus.

Disclosures: H. Koenig: None. **R.F. Schwamborn:** None. **J.H.M. Prehn:** None.

Nanosymposium

673. Metabolic and Excitotoxic Mechanisms of Cell Death and Degeneration

Location: 144A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:30 AM

Presentation Number: 673.05

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant NS084817

NIH Grant DA033966

Title: Pyramidal neurons in the medial prefrontal cortex of HIV-1 transgenic rats display over-excitation

Authors: ***C. KHODR**[1], S. DAVE*[1], L. AL-HARTHI[2,3], X.-T. HU[1,3];
[1]Dept. of Pharmacology, Ctr. for Compulsive Behavior and Addiction, [2]Dept. of Immunol. and Microbiology, Rush Univ. Med. Ctr., Chicago, IL; [3]The Developmental Ctr. for AIDS Res., Chicago, IL

Abstract: *These authors contributed equally. Human immunodeficiency virus-1 (HIV-1) infection can result in neurological and neuropsychiatric disorders, displaying neurocognitive, motor and behavioral impairments, which is also known as neuroAIDS. The medial prefrontal cortex (mPFC), a forebrain region that plays a key role in regulating cognitive, emotional and behavioral function, is altered by HIV. We previously demonstrated that acute exposure of rat brain slices to the HIV-1 protein Tat induces increased excitability of pyramidal neurons in the mPFC. In the current study, we evaluated the consequences of chronic, *in vivo* expression of HIV proteins on the neurophysiology of mPFC pyramidal neurons using HIV-1 transgenic (Tg) rats, which express seven of the nine HIV-1 proteins from the viral promoter. Five-six week old male non-Tg, control and HIV-1 Tg rats were used for electrophysiological assessment. Whole-cell patch-clamp recordings were performed on brain slices to determine functional activity of mPFC pyramidal neurons. We found that mPFC pyramidal neurons from HIV-1 Tg rats displayed abnormally-increased excitability, showing more depolarized resting membrane potentials (p=0.0016), reductions in the minimal depolarizing current required to elicit initial action potential (rheobase; p=0.0188), and increased firing in response to excitatory stimuli (p=0.0151) compared to those from non-Tg, control rats. Numerous abnormal firing properties, which were never seen in non-Tg, control rats, were also found in many neurons from HIV-1 Tg rats (68.75%; p=0.0009); such changes included reduced spike amplitude (p<0.0001), increased half-peak duration (p=0.0102), spontaneous firing, firing elicited by a post-hyperpolarization V_m rebound, and reduced firing with deformed or lack of action potentials. Further, the inward rectification was also significantly reduced in the neurons from HIV-1 Tg rats compared to control rats (p<0.001), suggesting a dysregulation in the subthreshold excitability. Overall, our findings indicate that persisting exposure to HIV-1 proteins *in vivo* renders mPFC pyramidal neurons more susceptible and vulnerable to excitatory stimuli, which may contribute to HIV neuropathogenesis.

Disclosures: **C. Khodr:** None. **S. Dave*:** None. **L. Al-Harthi:** None. **X. Hu:** None.

Nanosymposium

673. Metabolic and Excitotoxic Mechanisms of Cell Death and Degeneration

Location: 144A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:30 AM

Presentation Number: 673.06

Topic: C.08. Ischemia

Support: HL-077731

HL-093554

HL-065380

Title: Characterization of mitochondrial respiration in diazoxide preconditioned neurons

Authors: ***S. DUTTA**, I. RUTKAI, P. V. KATAKAM, D. W. BUSIJA;
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Abstract: Diazoxide (DZ) is a putative mitochondrial ATP sensitive potassium channel opener shown to have preconditioning effects in ischemic models in vitro and in vivo. Major initiating events include reactive oxygen species production, mitochondrial membrane depolarization, and phosphorylation of kinases such as protein kinase B (Akt), mammalian target of rapamycin (mTOR) and S6 kinase (S6K). To the best of our knowledge, no studies have been conducted on the influence of DZ preconditioning on mitochondrial respiration in intact neurons. We used E18 rat embryos to culture primary cortical neurons. On days in vitro (DIV) 7, 8, and 9 neurons were maintained in feeding medium or treated with DZ (500μM). On DIV 10, neurons were subjected to either 3 h oxygen glucose deprivation (OGD) or placed in an incubator for the same time period. After OGD, neurons were allowed a 24 h recovery period. For all groups, the Seahorse Xfe 24 analyzer was used to assess oxygen consumption rate using serial injections of oligomycin, carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP), and antimycin/rotenone (A/R). DZ-preconditioning significantly increased the percentage of neurons surviving after OGD (untreated OGD = 54.65±0.9%, DZ OGD = 64.83±2.3%, n=15 in each group). The mitochondrial respiratory response was not significantly different under normoxic conditions in vehicle or in DZ treated neurons. However, there was a significant increase in responses to each mitochondrial drug injected following OGD in DZ-treated neurons: oligomycin (OGD=47.98±1.7% [n=4], DZ OGD=57.66±2.4% [n= 4]); FCCP

(OGD=86.16±2.6% [n=4], DZ OGD=92.8±2.6% [n = 4]); A/R (OGD=29.82±1.7% [n=4], DZ OGD=37.75±2%[n=4]). This finding suggests there is an enhanced mitochondrial response after OGD with DZ preconditioning. Additionally, spare respiratory capacity showed a tendency to increase 24 h after OGD in DZ treated neurons. In conclusion, DZ enhances neuronal survival as well as mitochondrial respiration following OGD.

Disclosures: **S. Dutta:** None. **I. Rutkai:** None. **P.V. Katakam:** None. **D.W. Busija:** None.

Nanosymposium

673. Metabolic and Excitotoxic Mechanisms of Cell Death and Degeneration

Location: 144A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:30 AM

Presentation Number: 673.07

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: University of Eastern Finland Innovative Research Initiatives

Academy of Finland 127717

Academy of Finland 134893

Academy of Finland 135073

University of Eastern Finland Doctoral Programme of Molecular Medicine

Title: Recruitment of NOS1AP by nNOS contributes to activation of p38MAPK and cell death in models of excitotoxic neurodegeneration

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Abstract: Excitotoxicity is a multistep mechanism of neurodegeneration common to acute and chronic disorders of the nervous system. It is initiated by excess neurotransmitter glutamate and calcium entry through NMDA receptors (NRs), causing neurons to die. Interaction of calcium-activated neuronal nitric oxide synthase (nNOS) with NRs via PSD95 contributes to excitotoxicity, apparently via activation of p38MAPK cell death pathways. Direct inhibition of calcium influx and nNOS have not emerged as therapeutic approaches, but protein-protein interactions within the NR-PSD95-nNOS ternary complex have become increasingly attractive

targets. A peptide competing with the interaction of NR and the PDZ domains of PSD95 was the first successful neuroprotectant in stroke trials, while nNOS-derived peptides and small-molecule inhibitors devised to disrupt PSD95-nNOS interaction show in vitro and in vivo efficacy in models of excitotoxicity, pain and depression. Nevertheless, PSD-95 is a critical player at synapses and nNOS function should also be retained. For this reason we sought targets downstream of the NR-PSD95-nNOS ternary complex, upstream of p38MAPK, which might allow more selective neuroprotection. We identified a novel mediator of excitotoxic cell death, the nNOS ligand NOS1AP encoded by a gene associated with sudden cardiac death, diabetes, and schizophrenia. In resting cortical neurons, interaction of nNOS with NOS1AP is weak. This rapidly increases in parallel with p38MAPK activation in response to NMDA-evoked increase of intracellular [Ca2+]. NOS1AP forms a complex with p38MAPK activator, MKK3, and RNAi methods show that both NOS1AP and MKK3 are required for NMDA-evoked activation of p38. NMDA also induced interaction between nNOS and NOS1AP in organotypic hippocampal slice cultures. The ligand-binding pocket of nNOS that binds to NOS1AP is known to have unusual sequence specificity, which facilitated the development of a cell-permeable peptide TAT-GESV that disrupts nNOS-NOS1AP interaction. TAT-GESV interacts with the nNOS-ligand binding pocket and thus we find it selectively competes with NOS1AP but not with PSD95, nor does it detectably interact with PSD95-PDZ domains. Using this tool we were able to obtain neuroprotection in several models of excitotoxic neurodegeneration. In conclusion, the recruitment of NOS1AP by nNOS is acts as a mediator between activation of the NR-PSD95-nNOS complex signalling and downstream p38-dependent neurodegenerative signalling and may become a valuable basis for design of selective therapeutics for conditions involving aberrant NMDA receptor signalling.

Disclosures: **M.J. Courtney:** None. **W. Ba:** None. **N. Nadif Kasri:** None. **L. Li:** None.

Nanosymposium

673. Metabolic and Excitotoxic Mechanisms of Cell Death and Degeneration

Location: 144A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:30 AM

Presentation Number: 673.08

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Transgenic ß- Galactosidase expression in the brain is NOT inert for your mouse model

Authors: *J. M. REICHEL[1], B. BEDENK[1], C.-Y. KAO[2], C. TURCK[2], M. CZISCH[3], J. M. DEUSSING[1], C. T. WOTJAK[1];

[1]Stress Neurobio. and Neurogenetics, [2]Translational Res. of Psychiatry, [3]Core Unit Neuroimaging, Max Planck Inst. of Psychiatry, Munich, Germany

Abstract: Transgenic *lacZ* expression (i.e. ß- Galactosidase, ß- Gal) via the Rosa26 locus is a widely used tool to control for the activity and location of target genes. So far it has been believed to be inert itself; however, senescence associated ß- Galactosidase (SA- ß- Gal) and D-Galactosidase are both associated with an aged phenotype in mammals. Therefore we performed an in depth behavioral and structural (Manganese enhanced MRI, MEMRI) screening for several *lacZ* expressing mouse lines in order to ascertain whether ß- Gal is really inert to the resulting phenotype. We found that constitutive expression of *lacZ* under the control of the glutamatergic Nex promoter ($lacZ^{\mathbf{Nex+}}$) causes severe behavioral changes: increased locomotor activity, decreased anxiety, prominent cognitive impairment. Subsequent MEMRI scan revealed a 30% reduction in hippocampal volume. GFP^{Nex+} control mice or Cre^{Nex+} deleter mice did not show any of these alterations. However, $lacZ^{\mathbf{Dlx+}}$ (*lacZ* expression in GABAergic forebrain neurons), also showed increased locomotor activity, decreased anxiety and mild cognitive impairment, but no hippocampal volume reduction. Proteomic analysis of $lacZ^{\mathbf{Nex+}}$ hippocampal tissue punches also revealed a number of differentially regulated proteins in *lacZ* expressing mice. This led us to conclude that constitutive transgenic *lacZ* expression is NOT inert for the behavioral, structural (i.e. CNS) and molecular phenotype of the expressing organism. Both *Nex*- and *Dlx*-promoters are active during embryogenesis, therefore we also induced *lacZ* expression in adulthood under the control of several different promoters (CamKIIa, DAT, Nex) and found again prominent behavioral and structural changes – the specificity of each depending on the promoter. The resulting phenotype of $lacZ^{\mathbf{ERT2\text{-}Nex+}}$ mice mirrored the observed effects for $lacZ^{\mathbf{Nex+}}$ mice, albeit to a lesser extent. In conclusion, we demonstrate that transgenic *lacZ* expression in mice is NOT inert and in fact results in severe overall phenotypic changes. These changes are somewhat attenuated, but still significant, if *lacZ* expression is induced during adulthood.

Disclosures: J.M. Reichel: None. **B. Bedenk:** None. **C. Kao:** None. **C. Turck:** None. **M. Czisch:** None. **J.M. Deussing:** None. **C.T. Wotjak:** None.

Nanosymposium

673. Metabolic and Excitotoxic Mechanisms of Cell Death and Degeneration

Location: 144A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:30 AM

Presentation Number: 673.09

Topic: C.08. Ischemia

Support: NIH Grant NS67078 (PZ)

NIH Grant NS34179 (CI)

Title: The expression of the neuroprotective protein prohibitin during ischemic preconditioning requires nitric oxide

Authors: L. QIAN, C. J. ANDERSON, E.-M. PARK, G. MANFREDI, C. IADECOLA, *P. ZHOU;
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Abstract: Ischemic preconditioning (IPC) activates endogenous pathways for cell survival that lead to powerful neuroprotection in several brain injury models (Nat Neurosci 14:1363, 2011). IPC induced by short episodes of bilateral common carotid artery occlusion (BCCAO) requires nitric oxide (NO) derived from inducible NO synthase (iNOS) for its full expression (JCBFM 25:491, 2005). The mitochondrial protein prohibitin (PHB) is upregulated in brain tissue upon induction of IPC. Overexpression of PHB in neuronal cultures is protective against various injury modalities (J. Neurosci 32:583, 2012), whereas viral-mediated hippocampal PHB gene transfer attenuates CA1 injury in a mouse model of transient forebrain ischemia (Stroke 45:1131, 2014). We tested the hypothesis that the signaling events leading to PHB upregulation during IPC require NO. First we sought to determine whether the upregulation of PHB observed during IPC induced by BCCAO requires iNOS-derived NO. C57Bl6 mice were subjected to three episodes of 1-min BCCAO and sacrificed 24hrs later. BCCAO-induced IPC increased PHB protein expression, assessed by western blotting, an effect prevented by pretreatment with the iNOS inhibitor aminoguanidine (IPC: +31±4%, IPC+AG: +2±0.1%, n=4, $p<0.01$; mean±SE). To determine the mechanisms by which NO leads to PHB upregulation we established an in vitro model of NO mediated neuroprotection in primary neuronal cultures. Pre-treatment with the NO donor DPTA-NONOate (75μM) offered robust neuroprotection against oxygen glucose deprivation (OGD) (neuronal viability: vehicle: 25±3%; NO: 69±8%; n=5, $p<0.01$). The effect was associated with PHB upregulation (+47±5%, n=5, $p<0.05$ form vehicle). To determine whether the NO-soluble guanylate cyclase (sGC) signaling pathway is involved in PHB upregulation, we used the sGC inhibitor ODQ. However, the NO-induced enhancement in cell viability and PHB upregulation were not affected by ODQ pretreatment (dose range 0.1-10 μM; $p>0.05$, n=4) suggesting that the NO-sGC pathway may not be involved. The data indicate that endogenous NO production is required for the PHB upregulation induced by IPC and that exogenous NO can mimic the PHB upregulation afforded by IPC. The mechanisms of the effects of NO on PHB expression do not require sGC, but may involve other effects of NO, such as S-nitrosylation. The identification of the mechanisms by which NO upregulates PHB may unveil new approaches to increase the expression of this highly protective protein for the prevention or treatment of brain diseases. Support: NS34179 (CI), NS67078 (PZ)

Disclosures: **L. Qian:** None. **P. Zhou:** None. **C.J. Anderson:** None. **E. Park:** None. **G. Manfredi:** None. **C. Iadecola:** None.

Nanosymposium

673. Metabolic and Excitotoxic Mechanisms of Cell Death and Degeneration

Location: 144A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:30 AM

Presentation Number: 673.10

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH/NIMDS 5R01NS036761

Title: Geldanamycin induces FOXO3a-dependent motor neuron apoptosis

Authors: ***C. N. DENNYS**[1], A. STRAYER[2], M. FRANCO[1], A. G. ESTEVEZ[1];
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Abstract: Heat shock protein 90 (Hsp90) regulates numerous signaling pathways together with a variety of co-chaperones and over 300 client proteins. Geldanamycin, a specific Hsp90 inhibitor prevents apoptosis in various cell death models. Here we investigate the hypothesis that inhibition of Hsp90 by geldanamycin prevents trophic factor deprivation-induced motor neuron death. Hsp90 inhibition by geldanamycin stimulated motor neuron apoptosis (EC50 = 0.5 nM) in the presence of trophic factors. In fact, motor neurons incubated with trophic factors are 10-10,000 times more sensitive to Hsp90 inhibition by geldanamycin than trophic factor deprived motor neurons (EC50= 5nM) and other cell types including low and high-density primary cortical neurons (EC50 of 26.1 nM and 473.1 nM, respectively), dissociated spinal cords cultures (EC50 =146 nM), and differentiated and undifferentiated NSC34 (EC50 of 800 nM and 980 nM respectively, $p<0.05$). Caspase inhibitors prevented geldanamycin-induced motor neuron apoptosis ($p<0.001$). The Fas receptor decoy Fas:Fc increased resistance of motor neurons to geldanamycin induced cell death (EC50 of 5 nM, $p<0.001$). Geldanamycin stimulated Fas ligand expression as determined by qPCR ($p<0.001$), which was preceded by translocation of FOXO3a to the nucleus. A luciferase reporter under the regulation of forkhead responsive element (FHRE) was induced following 16 hours geldanamycin treatment. Overexpression of constitutively active PDK1, PI3K and AKT prevented geldanamycin-induced motor neuron apoptosis ($p<0.05$). These results suggest that motor neurons sensitivity to geldanamycin is due to inhibition of the PI3K/AKT pathway, leading to FOXO3a-dependent expression of Fas ligand.

Disclosures: **C.N. Dennys:** None. **A. Strayer:** None. **M. Franco:** None. **A.G. Estevez:** None.

Nanosymposium

673. Metabolic and Excitotoxic Mechanisms of Cell Death and Degeneration

Location: 144A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:30 AM

Presentation Number: 673.11

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Health Research Board in Ireland Grant PhD/2007/11

Science Foundation Ireland Grant 08/IN.1/B1949

Title: Single-cell imaging of bioenergetic responses to neuronal excitotoxicity and oxygen and glucose deprivation

Authors: ***N. M. CONNOLLY**[1], H. DUESSMANN[1], U. ANILKUMAR[1], H. J. HUBER[2], J. H. M. PREHN[1];
[1]Dept. of Physiol. & Med. Physics, Royal Col. of Surgeons In Ireland, Dublin 2, Ireland; [2]Dept. of Cardiovasc. Sci., KU Leuven, Leuven, Belgium

Abstract: Excitotoxicity is a condition occurring during cerebral ischaemia, seizures, and chronic neurodegeneration. It is characterised by over-activation of glutamate receptors, leading to excessive Ca2+/Na+ influx into neurons, energetic stress and subsequent neuronal injury. We and others have previously investigated neuronal populations to study how bioenergetic parameters determine neuronal injury, however such experiments are often confounded by population-based heterogeneity and contribution of effects of non-neuronal cells. Hence, we here characterised bioenergetics during transient excitotoxicity in primary neurons at the single-cell level using fluorescent sensors for intracellular glucose, ATP, and activation of the energy sensor AMP-activated protein Kinase (AMPK). We identified ATP depletion and recovery to energetic homeostasis, along with AMPK activation, as surprisingly rapid and plastic responses in two excitotoxic injury paradigms. We observed rapid recovery of neuronal ATP levels also in the absence of extracellular glucose, or when glycolytic ATP production was inhibited, but found mitochondria to be critical for fast and complete energetic recovery. Employing an injury model of oxygen and glucose deprivation, we identified a similarly rapid bioenergetics response, yet with incomplete ATP recovery and decreased AMPK activity. Interestingly, excitotoxicity also induced an accumulation of intracellular glucose, providing an additional source of energy during and after excitotoxicity-induced energy depletion. We identified this to originate from

extracellular, AMPK-dependent glucose uptake and from intracellular glucose mobilisation. Surprisingly, cells recovering their elevated glucose levels faster to baseline survived longer, indicating that the plasticity of neurons to adapt to bioenergetic challenges is a key indicator of neuronal viability.

Disclosures: **N.M. Connolly:** None. **H. Duessmann:** None. **U. Anilkumar:** None. **H.J. Huber:** None. **J.H.M. Prehn:** None.

Nanosymposium

673. Metabolic and Excitotoxic Mechanisms of Cell Death and Degeneration

Location: 144A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:30 AM

Presentation Number: 673.12

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Air Force Office of Scientific Research (London), Air Force Material Command, USAF, under grant number FA8655-05-1-3065

Air Force Material Command, USAF, under grant number FA8655-05-1-3065

Swedish Medical Research Council Grant Nr 2710

Title: Nanomedicine for Central Nervous System: Polymeric nanoparticles for therapeutic strategies in neurological disorders

Authors: *G. TOSI[1], B. RUOZI[2], D. BELLETTI[3], P. VERATTI[3], F. PEDERZOLI[3], M. ZOLI[4], A. VILELLA[4], A. M. GRABRUCKER[5], H. S. SHARMA[6], A. SHARMA[7], M. A. VANDELLI[3], F. FORNI[3];
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Abstract: Non-invasive strategies for treatment of Central Nervous System (CNS) diseases based on colloidal carriers represent a huge potential to efficiently transport drug across the Blood Brain Barrier (BBB), since nanocarriers can protect drugs (or gene material) and deliver them to target specific populations of brain cells. Thus, liposomes and polymeric nanoparticles

(NPs) engineered on their surface with ligands, antibodies or peptides are currently under investigation as they could be considered as innovative tools for achieving CNS drug delivery. Besides a short overview on the recent literature results of nanomedicine applied to brain diseases, the presentation will particularly focus on glycopeptide-modified NPs (g7-NPs) able to target the CNS as emphasized by in vivo investigation. After administration by different routes (i.v./i.p./oral/nasal), g7-NPs showed a remarkable capability to reach the CNS (up to 10% of the injected dose) in rodent animal models along with proof-of-evidences based on biodistribution and pharmacological studies. Inhibition studies both in vivo and in vitro highlighted the brain localization and, as preferential pathway, the clathrin-mediated endocytosis [1]. However, the fate of g7-NPs in the brain remained an open question. Thus, we tried to answer to some issues as dose/time-dependent accumulation, the tropism to specific brain regions and cell sub-populations identifying a correlation between in vivo and in vitro uptake mechanism in neurons [2]. In conclusion, several outcomes highlighted the efficiency of g7-NPs in brain targeting together with a deep explanation of the fate and the trafficking of these kind of NPs after BBB crossing. References: 1.Tosi, G.; Vilella, A.; Chhabra, R.;, Schmeisser, M.J.; Boeckers, T.M.; Ruozi, B.; Vandelli, M.A.; Forni, F.; Zoli, M.; Grabrucker. A.M.. Insight on the fate of CNS-targeted nanoparticles. Part II: Intercellular neuronal cell-to-cell transport. J Control Release. 2014, 177, 96-107. 2.Vilella, A.; Tosi, G.; Grabrucker, A.M.3; Ruozi, B.; Belletti, D.; Vandelli, M.A.; Boeckers, T.M.; Forni, F.; Zoli, M. Insight on the fate of CNS-targeted nanoparticles. Part I: Rab5-dependent cell-specific uptake and distribution. J Control Release. 2014, 174, 195-201.

Disclosures: G. Tosi: None. **B. Ruozi:** None. **D. Belletti:** None. **P. Veratti:** None. **F. Pederzoli:** None. **M. Zoli:** None. **A. Vilella:** None. **A.M. Grabrucker:** None. **H.S. Sharma:** None. **A. Sharma:** None. **M.A. Vandelli:** None. **F. Forni:** None.

Nanosymposium

673. Metabolic and Excitotoxic Mechanisms of Cell Death and Degeneration

Location: 144A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:30 AM

Presentation Number: 673.13

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01 NS056049

NIH Grant P50 AG008702

Title: The role of Vps34 and its product PI3P in neuronal autophagy and tau clearance

Authors: *Z. M. LASIECKA, K. E. DUFF, S. A. SMALL, G. DI PAOLO;
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Abstract: Lipid signaling controls a myriad of cellular processes, ranging from membrane trafficking to signal transduction and as such are increasingly linked to human disease. Alzheimer's disease (AD) is one such disorder in which lipid dyshomeostasis and membrane trafficking defects are believed to play a critical role. We have recently conducted a lipidomic analysis of brain samples derived from transgenic animal models of familial AD as well as brain regions from patients with late-onset AD. Levels of phosphatidylinositol-3-phosphate (PI3P) were significantly reduced in AD-affected brain regions in mice and men. PI3P is a phosphoinositide primarily synthesized by lipid kinase Vps34 (*i.e.*, class III phosphatidylinositol 3-kinase) and acts as a master regulator of the endosomal and autophagy pathways. Knocking down/out Vps34 was found to recapitulate salient features linked to late onset AD pathogenesis, namely enlarged endosomes and aberrant endosomal trafficking and amyloidogenic processing of the amyloid precursor protein (APP). In this study, we focus on the effect of Vps34 ablation and PI3P deficiency on neuronal autophagy and on autophagic clearance of tau aggregates. Autophagy is an essential cellular pathway mediating the lysosomal degradation of defective organelles, long-lived proteins, and a variety of protein aggregates through the formation of autophagosomes, which sequester cytoplasmic cargoes prior to their fusion with lysosomes. Accordingly, perturbation of autophagy has been linked to various neurodegenerative disorders, including AD. Ablation of Vps34 in *Vps34*$^{Flox/Flox}$ mouse hippocampal neurons, using lentivirus expressing Cre recombinase, lead to reduced LC3 immunoreactivity, suggesting the presence of fewer autophagosomes, as well as diminished cellular response to both stimulation of autophagy and blockade of autophagic clearance. Furthermore, we observed a dramatic increase in p62 levels and immunoreactivity in mutant cultured neurons, denoting the accumulation of non-digested autophagy cargos. Additionally, we investigated the effect of Vps34 ablation *in vivo* in the brain of *CamKIIalpha; Vps34*$^{Flox/Flox}$ mice. Neuronal loss, gliosis as well as p62 accumulation were found in the cortex, hippocampus and dentate gyrus of 3 months old mice. Furthermore, we observed anti-phospho-tau immunoreactivity reminiscent of aggregates in the CA1 region of hippocampus as well as the cortex of 4 months old *CamKIIalpha Cre; Vps34*$^{Flox/Flox}$*; hTau; mTau*$^{-/-}$ mice. Overall, our data indicate that lack of Vps34 reduces the efficacy of functional autophagy in neuronal cultures and *in vivo*, possibly promoting aberrant tau accumulation.

Disclosures: Z.M. Lasiecka: None. **K.E. Duff:** None. **S.A. Small:** None. **G. Di Paolo:** None.

Nanosymposium

673. Metabolic and Excitotoxic Mechanisms of Cell Death and Degeneration

Location: 144A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:30 AM

Presentation Number: 673.14

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 1K99NS082619 to SM

NIH Grant NS060698 to EH

Title: Autophagosome biogenesis in primary neurons follows an ordered and spatially regulated pathway

Authors: *S. MADAY, E. L. F. HOLZBAUR;
Dept. of Physiol., Univ. of Pennsylvania Sch. of Med., Philadelphia, PA

Abstract: Autophagy is an essential lysosomal degradation pathway in neurons, yet little is known about the mechanisms driving this pathway in these highly polarized cells. In primary dorsal root ganglion (DRG) neurons, we have shown that autophagosomes are constitutively generated in the distal axon and undergo robust transport toward the cell soma. As they move distally to proximally, autophagosomes mature into autolysosomes that more effectively catalyze cargo degradation. Here, we use dual-color live-cell imaging to investigate the neuron-specific mechanisms of autophagosome biogenesis under basal conditions. Puncta positive for the autophagosome-assembly factors Atg13 and Atg5 appear almost exclusively in the distal axon and grow progressively in size. LC3 is recruited to these nascent autophagosomes, Atg13 and Atg5 dissociate, and the LC3 puncta then grow progressively into ring structures ~800 nm in diameter. Quantitative analysis of the temporal dynamics reveals that this ordered recruitment of assembly factors proceeds with stereotypical kinetics. During formation, we did not observe incorporation of plasma- or mitochondrial-derived membrane into nascent autophagosomes in the distal axon. Rather, autophagosomes are generated at subdomains of the endoplasmic reticulum positive for DFCP1 and distinct from ER exit sites. Remarkably, these biogenesis events are highly enriched in the distal axon, with rates of formation ~20-fold higher than observed along the mid-axon. Autophagosomes form infrequently in the cell soma or mid-axon, indicating that this process is spatially enriched in the distal axon, consistent with a highly compartmentalized pathway for constitutive autophagy in primary neurons. This overall paradigm of distal initiation followed by robust retrograde transport is not limited to developing DRG neurons and is also observed in synaptically-connected hippocampal neurons. We propose that distal enrichment of autophagosome formation facilitates the degradation of damaged mitochondria and long-lived cytoplasmic proteins that reach the axon tip via slow axonal transport. Supported by NIH 1K99NS082619 to SM and NIH NS060698 to EH.

Disclosures: S. Maday: None. **E.L.F. Holzbaur:** None.

Nanosymposium

674. Extrastriate Cortex: Neural Coding

Location: 143A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:30 AM

Presentation Number: 674.01

Topic: D.04. Vision

Support: NHMRC APP1008287

HFSP CDA00029

Title: Population coding of motion direction in marmoset area MT is rapid and sustained

Authors: *E. ZAVITZ, S. HAGHGOOIE, H.-H. YU, A. J. DAVIES, M. G. P. ROSA, N. S. C. PRICE;
Physiol., Monash Univ., Clayton, Australia

Abstract: Introduction: The rapid detection and characterization of motion is one of the early visual system's most critical tasks. Neurons in the primate middle temporal area (MT) are known to provide robust, direction selective, responses to moving stimuli. Here, we investigated the reliability with which motion direction can be identified on short and long timescales from the spiking activity of a population of MT neurons. **Methods:** We recorded spiking activity from 96-electrode "Utah" arrays implanted in area MT of two anaesthetised marmosets (*Callithrix jacchus*). Neural responses were recorded while a dot stimulus that moved coherently in a single direction was presented. We used two types of dot stimuli to examine decoding on both long and short timescales. The slow-timescale stimulus consisted of white dots on a black field and that moved coherently in one of 12 directions for 500 ms, followed by 500 ms of a blank screen. The fast-timescale stimulus consisted of a black and white dots on a grey field that moved coherently in one of 24 directions for one to four frames (8.3 to 33.2 ms at 120 Hz) in a continuous sequence of directions with no blank periods. To evaluate coding, we randomly split the data into training (90% of trials) and testing (10% of trials) sets. We then fit a series of generalized linear models to the training data at many time lags to predict the stimulus direction at each lag (10 ms resolution) based on tuned multiunit neural activity recorded from 54 (Animal 1) or 72 (Animal 2) electrodes. To measure decoding performance, we generated model predictions from the remaining test data. **Results:** For the slow-timescale stimulus, we found that decoding performance was above chance (1/12 = 8.3%) from 70 ms after stimulus onset and reached a peak of up to 80% correct. For the fast-timescale stimulus, stimulus direction could be reliably predicted using just 30 ms of spiking data for each channel (peak performance 27% correct; chance=12.5%). **Conclusions:** Even using a small subset of the neural population, we were able

to decode stimulus direction from the population activity. Our results suggest: (1) that the neural activity in area MT provides enough information for a reliable prediction of stimulus direction less than 100ms after stimulus onset, and (2) that the population code retains the stimulus direction for more than 100ms after stimulus offset, even in the presence of further visual stimulation, providing some 'memory' of direction.

Disclosures: **E. Zavitz:** None. **S. Haghgooie:** None. **H. Yu:** None. **A.J. Davies:** None. **M.G.P. Rosa:** None. **N.S.C. Price:** None.

Nanosymposium

674. Extrastriate Cortex: Neural Coding

Location: 143A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:30 AM

Presentation Number: 674.02

Topic: D.04. Vision

Support: NIH Grant EY04440

FWO Postdoctoral Fellowship

HHMI

Title: Separability of spatiotemporal receptive field structure in macaque area MT

Authors: ***A. D. ZAHARIA**, R. L. T. GORIS, J. A. MOVSHON, E. P. SIMONCELLI; Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: What determines the shape of the spatiotemporal tuning curves of visual neurons? In V1, direction-selective simple cells have selectivity that is separable in orientation, spatial frequency, and temporal frequency ("frequency separable"). In contrast, many V1 direction-selective complex cells and MT cells are non-separably tuned along these axes, but are tuned for speed instead. Furthermore, MT neurons show narrower speed tuning to random dot stimuli than to sinusoidal gratings, suggesting a more invariant representation of speed. Some models for tuning in area MT predict that signals from V1 inputs with linked spatial and temporal frequency selectivity are combined to create tuning curves organized around tilted planes, each representing stimuli translating at a particular direction and speed ("velocity separable"). Goris et al (SfN, 2012) examined the organization of spatiotemporal frequency selectivity as revealed by MT neuron responses to sinusoidal gratings whose drift direction varied with drift rate either held constant (frequency separable organization) or varying along the preferred velocity plane

(velocity separable). Some MT neurons' tuning was better fit by a frequency separable model, while others were better fit by a velocity separable model. There was no clear relationship between the degree of pattern selectivity and the degree to which the velocity separable model better described the neuron's tuning. Because MT neurons can respond differently to superpositions of gratings than expected from the responses to the component gratings measured in isolation, we extended this study to include plaids (sums of two gratings with orientation differing by 120º). Frequency separable plaids were constructed from gratings moving at the preferred drift rate of the cell. In contrast, the drift rates of components of velocity separable plaids were chosen to lie in the preferred velocity plane, as in the single grating experiment. MT responses to velocity separable plaids were significantly stronger than those to frequency separable plaids. Moreover, pattern cells had a broader bandwidth on the velocity plane than component cells. Both of these observations are consistent with predictions of the velocity separable model. We conclude that use of stimuli organized in a velocity separable coordinate system allows for a more refined dissection of the relationship between pattern selectivity and the degree to which spatiotemporal selectivity is organized with respect to the preferred velocity plane.

Disclosures: A.D. Zaharia: None. **R.L.T. Goris:** None. **J.A. Movshon:** None. **E.P. Simoncelli:** None.

Nanosymposium

674. Extrastriate Cortex: Neural Coding

Location: 143A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:30 AM

Presentation Number: 674.03

Topic: D.04. Vision

Support: NIH 1RO1-EY018897

NEI Core Grant EY-01876

Title: Orientation and hue maps in V4: Features and interactions

Authors: *D. J. FELLEMAN[1], A. PARAJULI[2];
[1]NBA, Univ. Texas Med. Sch., HOUSTON, TX; [2]Neurobio. and Anat., Univ. of Texas Med. School-Houston, Houston, TX

Abstract: Area V4 is an ‘intermediate’ cortical area that is suggested to play a critical role in color and shape processing due to its selectivity for relatively complex shapes and chromatic interactions. We used intrinsic imaging to determine (1) the degree to which color- and orientation-preferring domains are restricted to separate anterior/posterior positions in V4, (2) how maps of orientation and color are organized in V4, and (3) how oriented contours defined by luminance or chromatic contrast are represented in V4. Functional images were acquired using a slow-scan CCD camera in Sufentanil-anesthetized macaque monkeys. The overall patterns of orientation and hue responses were assessed at low magnification (7 mm field of view; 21 μm/pixel) and at higher magnification (9 μm/pixel) to study their detailed features. Orientation and hue maps were computed through the vector summation of single-condition responses elicited by 4-8 luminance or chromatic contrast oriented stimuli. Iso-luminant chromatic stimuli were selected using 4-8 equally spaced color angles (CIE luv 1976) to insure balanced and complete hue coverage. In some cases, these same color angle stimuli were presented at two different luminances. In agreement with Tanigawa et al. (2010), we observed spatially organized orientation and hue domains across parafoveal V4. However, although these different domains were somewhat spatially segregated, they did not exhibit an obvious anterior/posterior segregation on the prelunate gyrus. The representation of orientation in highly selective portions of V4 was variable; some maps formed clear ~ 1 mm orientation pinwheel while other maps were more elongated. Similarly, the representation of hue in highly selective portions of V4 often were organized into .5-1 mm hue pinwheel maps, while in others hue domains were more elongated. Furthermore, these hue maps retained their organizations despite changes in the luminance (2.5 and 7 cd/m2). Several different types of interactions between orientation and hue were observed: (1) regions highly selective for orientation and hue often overlapped just outside of hue pinwheels, (2) orientation maps defined by luminance or chromatic contrast activated different, yet nearby portions of V4 that were distinct from the hue maps themselves. These data suggest that V4 the representation of orientation and hue in area V4 is similar to that in area V2 in some respected, but differs substantially in the types of and degree of interactions between orientation and hue. Furthermore, these representation are more variable in form as compared to V1 and V2, suggesting they emerge through a more experience-dependent process.

Disclosures: D.J. Felleman: None. **A. Parajuli:** None.

Nanosymposium

674. Extrastriate Cortex: Neural Coding

Location: 143A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:30 AM

Presentation Number: 674.04

Topic: D.04. Vision

Support: NWO Vici grant awarded to PRR

FP7 Project 269921 “BrainScaleS"

Title: Microstimulation in V1 and V4 reveals asymmetric feedforward and feedback influences in texture-segregation

Authors: *P. R. ROELFSEMA, M.-A. GARIEL-MATHIS, B. DAGNINO;
Vision and Cognition, Netherlands Inst. for Neurosci., Amsterdam, Netherlands

Abstract: A central question in systems neuroscience is how lower and higher areas of the visual cortex work together during visual perception. Theories propose that feedforward connections from lower to higher brain areas drive neurons, whereas feedback connections serve to amplify the relevant visual feature representations while suppressing the representation of features that are task-irrelevant (Sherman & Guillery, PNAS, 1998; Roelfsema, Annu. Rev. Neurosci., 2006). However, evidence for this asymmetry in the feedforward and feedback effects is sparse. To gain insight into the properties of feedforward and feedback connections, we combined microstimulation with multi-unit recordings in areas V1 and V4 of the visual cortex of macaque monkeys performing a texture-segregation task. Microstimulation can cause orthodromic and antidromic stimulation effects, but we took a number of measures to minimize the antidromic contribution: (i) using current levels that cause most neurons to be stimulated trans-synaptically; (ii) stimulating V1 and V4, with relatively sparse direct connectivity; (iii) directly comparing of V1 and V4 microstimulation as an internal control. (1) Feedforward effects: In accordance with previous work (Butovas & Schwartz, J. Neurophysiol. 2003) a 20ms train of microstimulation caused a brief excitation in the stimulated brain region followed by longer inhibitory phase. V1 microstimulation caused an excitatory phase that drove V4 neurons, even in the absence of a visual stimulus. V1 microstimulation preceding the visual stimulus decreased visually driven activity in V4, suggesting that microstimulation activated the same feedforward connections as the visual stimulus did. (2) Feedback effects: V4 stimulation did not influence V1 activity in the absence of a visual stimulus. However, if V1 neurons were activated by a visual stimulus, V4 stimulation decreased V1 activity during the phase where the V4 activity itself was suppressed due to the delayed inhibition, indicating that the delayed V1 response depended on feedback from V4. The suppressive effect of V4 stimulation was strongest if V1 receptive fields fell on a texture-defined figure and weak if they fell on the textured background, implying that the extra activity elicited by texture-defined figures requires feedback from V4. These results provide unprecedented insight into the asymmetry of feedforward and feedback influences during visual perception. Feedforward connections drive activity in higher areas, whereas feedback connections modulate the activity of only those neurons that are driven by a visual stimulus.

Disclosures: **P.R. Roelfsema:** None. **M. Gariel-Mathis:** None. **B. Dagnino:** None.

Nanosymposium

674. Extrastriate Cortex: Neural Coding

Location: 143A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:30 AM

Presentation Number: 674.05

Topic: D.04. Vision

Support: The Gatsby Charitable Foundation

Title: Differences between central and peripheral visual fields' feedforward and feedback neural connections: Theoretical and empirical studies of perceptual decoding

Authors: *L. ZHAOPING;
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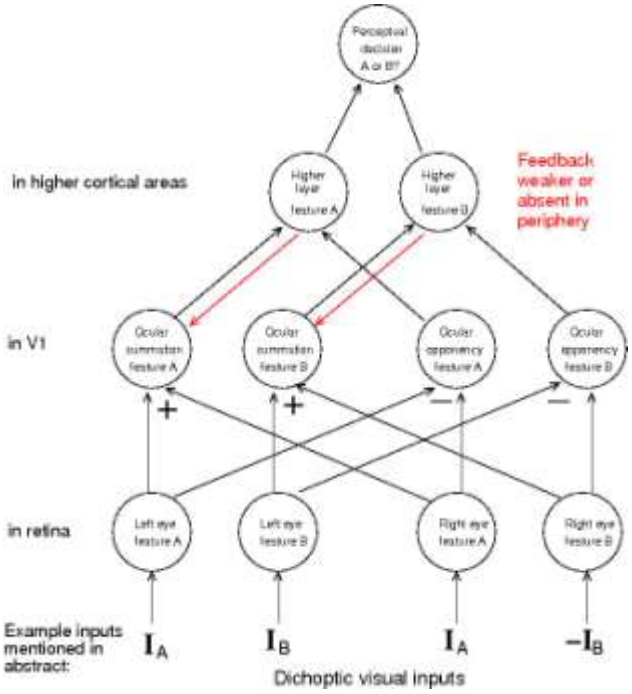
Abstract: Inputs from the peripheral visual field are coded mainly, or only, in lower visual cortical areas like V1, rather than higher cortical areas like V4 and IT. I report findings suggesting that top-down feedback from higher to lower cortical areas for the computation of analysis (recognition) by input synthesis is weaker or absent in the visual periphery. This feedback is expected to boost processing of inputs that agree with the higher area's interpretation of inputs, favoring likely patterns of input, for instance correlated, rather than anti-correlated, input between the two eyes. We exploited this ocular asymmetry in feedback by combining patterns A and B (gratings differing in drift direction or orientation) to show A+B to one eye and A-B to the other, making A and B represented in V1 in ocular summation and ocular opponent channels, respectively (Li and Atick, 1994). A and B would rival perceptually, giving an ambiguous percept of drift direction or orientation. The summation pattern (A) should be perceptually favored by feedback; this was observed, but to a lesser degree or not at all when inputs were shown in the periphery (10 degrees eccentricity, inputs enlarged to suit visual acuity, Zhaoping 2013). I introduce a circuit model of the cortical hierarchy. In the model, visual inputs are received by detectors for any feature (A or B) in both the ocular summation and ocular opponent channels in the V1 layer. Feature neurons in the higher cortical layer favoring A or B receive feed-forward signals about their preferred feature from both the ocular summation and opponent channels, but send reinforcing feedback only to the corresponding feature detector neurons in the summation channel, and only, or at least mainly, to neurons covering the central visual field. For analysis by synthesis, this feedback boosts the activation in the ocular summation channel, favoring visual input to this channel in a rivalry with visual input to the ocular opponent channel. This model is consistent with the finding that the central bias is stronger when input is viewed longer or moving more slowly to aid top-down scrutiny.



Disclosures: L. Zhaoping: None.

Nanosymposium

674. Extrastriate Cortex: Neural Coding

Location: 143A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:30 AM

Presentation Number: 674.06

Topic: D.04. Vision

Support: ONR-MURI Grant N000141010278

NIH Grant R01-EY002966

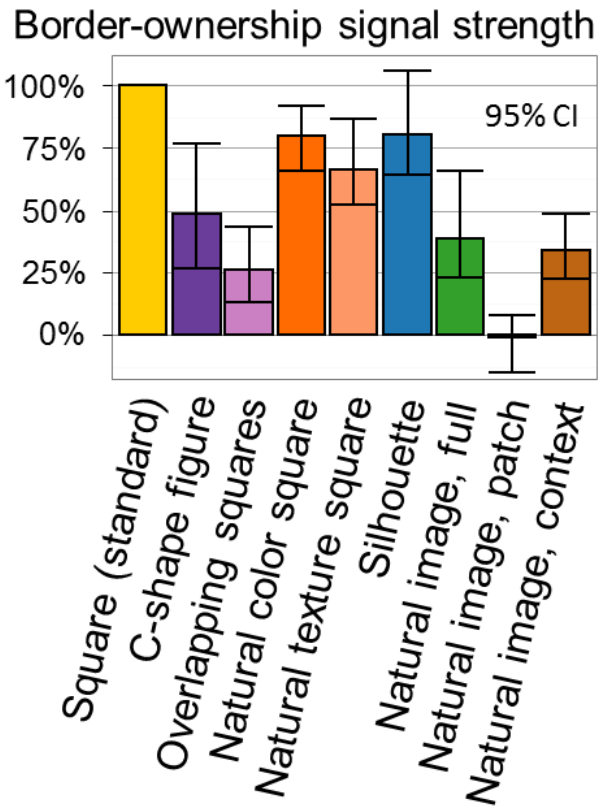
NIH Grant R01-EY016281

NIH Grant T32-EYT07143

Title: Early visual cortex consistently estimates border-ownership in simple figures and natural scenes

Authors: *J. WILLIFORD[1], R. VON DER HEYDT[1,2];
[1]Neurosci. Department, Sch. of Med., [2]Krieger Mind/Brain Inst., Johns Hopkins Univ., Baltimore, MD

Abstract: Discerning objects from their backgrounds is a fundamental process of vision and is necessary for visual perception. The coding of border-ownership in the early visual cortex is a neural correlate of this process. When stimulated with the contour of a figure, border-ownership selective neurons respond more strongly when the figure is on one side of their receptive field (the "preferred" side) versus the other (Williford & von der Heydt: Scholarpedia 8(10):30040, 2013). So far, border-ownership coding has only been shown with simple displays of geometric shapes (e.g., squares). Here we studied border-ownership coding with static images of natural scenes, geometric shapes, and stimuli that are "hybrids" of natural and geometric shapes. We used microelectrodes to record from isolated neurons in V1 and V2 of macaques. We found that subsets of V1 and V2 neurons indeed code for border-ownership in complex natural scenes. Decomposition of local and context influences showed that the context-based border-ownership signals correlated with those for the (locally ambiguous) edge of a square, but were one-third as strong on average. We used stimuli with intermediate complexity along several dimensions to measure the relative influences of object shape, occlusion between objects, texture and color contrast to determine how they contribute to the border-ownership signal strength. We found that border-ownership signal decreases with the stimulus complexity. This was especially pronounced when comparing the edge of a square with the concave edge of a C-shape, the border between overlapping squares, and contours of natural objects. The complexity of the silhouette shapes were also inversely correlated with the strength of the border-ownership signal. The context-based signal for natural scenes emerged at about 70 ms after stimulus onset and plateaued before 200 ms. In conclusion, subsets of neurons in V1 and V2 code for the border-ownership in natural scenes, however, the strength and accuracy of these early estimates of border-ownership decreases with the complexity of the visual stimulus.



Disclosures: **J. Williford:** None. **R. von der Heydt:** None.

Nanosymposium

674. Extrastriate Cortex: Neural Coding

Location: 143A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:30 AM

Presentation Number: 674.07

Topic: D.04. Vision

Support: Grant-in-Aid for Specially Promoted Research from MEXT to Y.M. (19002010 and 24220008)

CREST grant from Japan Science and Technology Agency to Y.M.

Grant from Takeda Science Foundation to Y.M.

Grant-in-Aid for Young Scientists (B) from MEXT to T.H. (25870142)

Grant-in-Aid for Young Scientists (B) from MEXT to K.W.K. (25830001)

Title: Microcircuits for hierarchical coding of object-object association across inferotemporal areas of macaques

Authors: *T. HIRABAYASHI[1], K. TAMURA[1], D. TAKEUCHI[1], M. TAKEDA[1], K. W. KOYANO[1], Y. MIYASHITA[1,2,3];
[1]Dept. of Physiol., The Univ. Tokyo Sch. Med., Tokyo, Japan; [2]CREST, JST, Saitama, Japan; [3]CNSI, NINS, Tokyo, Japan

Abstract: In primates, neuronal representations of visual objects are processed hierarchically in occipito-temporal cortices. In this hierarchy, representation of a "novel" object feature has been thought to emerge and become prevalent within a single cortical area as a result of the processing occurred in that area. Here, we tested another possibility that a feature representation prevalent in a given area emerges in the microcircuit of a hierarchically prior area as a small number of prototypes, and then becomes prevalent in the subsequent area. We trained monkeys to perform an object-object association task, and recorded multiple single-unit activities in each of hierarchically successive areas TE and 36 of the macaque inferotemporal (IT) cortex, during which monkeys performed this task. Directed neuronal coupling was examined by calculating cross-correlograms between single-unit activities that encoded learned objects. In area TE, the lower-order area, directed coupling was predominantly observed from neurons coding for an individual object to neurons coding for a learned pair of objects, suggesting the existence of predicted convergent microcircuit that generates the representation of object-object association. In area 36, the higher-order area, such convergent microcircuit was not observed, but instead, associative codes were gradually built up in the microcircuit during the course of cue presentation. These results suggest a computational principle underlying sequentially elaborated object representations across successive stages in the macaque IT cortex.

Disclosures: T. Hirabayashi: None. **K. Tamura:** None. **D. Takeuchi:** None. **M. Takeda:** None. **K.W. Koyano:** None. **Y. Miyashita:** None.

Nanosymposium

674. Extrastriate Cortex: Neural Coding

Location: 143A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:30 AM

Presentation Number: 674.08

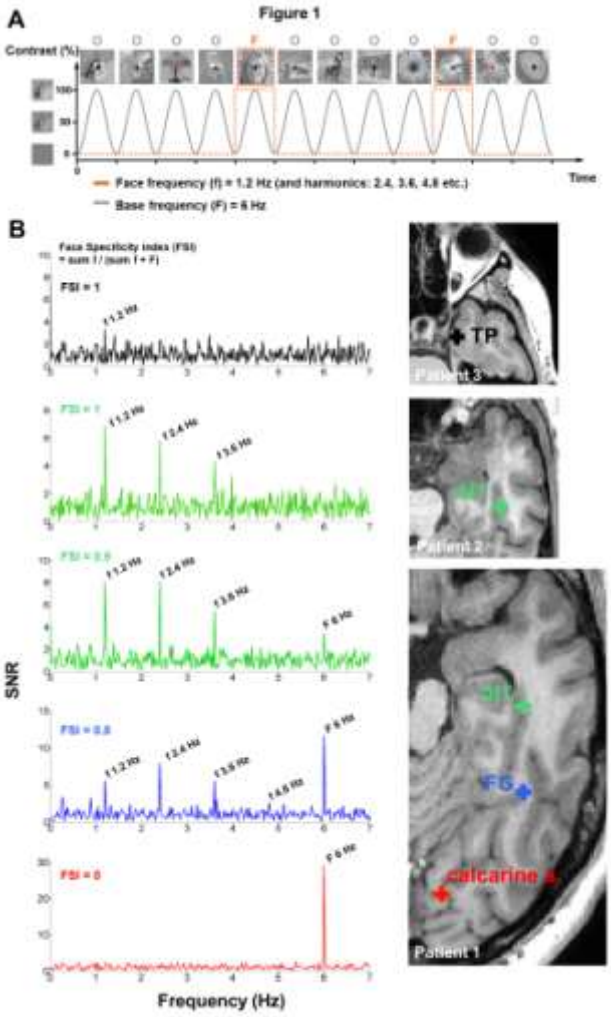
Topic: D.04. Vision

Support: ERC grant facessvep 284025

Title: A gradual increase of face-selectivity along the human ventral visual pathway: direct evidence from intracerebral recordings with fast periodic visual stimulation

Authors: *J. JONAS[1,2], J. LIU-SHUANG[2], C. JACQUES[2], L. MAILLARD[1], B. ROSSION[2]; [1]Service de Neurologie, Hop. Central, CHU de Nancy, Nancy, France; [2]Catholic Univ. of Louvain (UCL), Louvain-la-Neuve, Belgium

Abstract: Face perception involves a large set of regions distributed along the ventral temporal cortex and thus represents an interesting model to help understand how visual information is processed along the ventral visual pathway. Here we shed light on the functional organization of this cortical network by recording focal intracerebral electroencephalogram in epileptic patients implanted with depth electrodes. We objectively defined and quantified face-selective responses in the frequency domain by means of an original fast periodic visual stimulation approach (Liu-Shuang et al., 2014). Fifteen patients were presented with various object categories (houses, animals, plants, etc.) at a base frequency of 6 Hz (6 images/second) with variable face stimuli interleaved in this sequence at regular intervals of 5 stimuli (face frequency = 6 Hz/5 = 1.2 Hz) (see Figure 1A). Patients were only instructed to detect colour changes of the fixation cross. Robust and task-free face-selective responses occurring exactly at the 1.2 Hz oddball frequency and its harmonics (2.4, 3.6, etc.) were found in the lateral occpital cortex and in the fusiform gyrus (FG), but also in the antero-inferior temporal cortex (AIT: anterior collateral and occipito-temporal sulci) and in the temporal pole (TP). Interestingly, there was a posterior-anterior gradient of face-specificity: face/base frequency ratio increased along the ventral temporal regions (see Figure 1B for typical responses in 3 patients). Although the magnitude of the face-selective responses were the largest in regions that also responded to all visual stimuli (FG), “pure” response to faces (oddball responses in the absence of any 6 Hz general visual responses) were observed only in the AIT and in the TP (see patients 2 and 3 in Figure 1B). These findings point to a sharpening of face-selectivity along the ventral visual pathway and reveal that exclusive response to faces can be found at a macroscopic (cell populations) level of cortical organization.



Disclosures: **J. Jonas:** None. **J. Liu-Shuang:** None. **C. Jacques:** None. **L. Maillard:** None. **B. Rossion:** None.

Nanosymposium

674. Extrastriate Cortex: Neural Coding

Location: 143A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:30 AM

Presentation Number: 674.09

Topic: D.04. Vision

Support: NIH Grant EY019493

NSF CAREER grant 1254123

McKnight Scholar Award

Ray Thomas Edwards Career Award

Title: Organizing principles of phase-invariant selectivity along curved contours in mid-level vision

Authors: *T. O. SHARPEE, R. J. ROWEKAMP;
The Salk Inst. For Biol. Studies, LA JOLLA, CA

Abstract: As visual signals are processed at successive stages of visual processing, neurons pool information from larger sections of the visual space and also signal the presence of increasing complex visual features. Both of these properties increase the number of stimulus features that modulate a neuron's response. Here report the results of analysis of responses of neurons in extrastriate cortical areas of awake animals that viewed natural spatiotemporal scenes during passive fixation tasks. The neural responses were analyzed using a framework that models a neuron's response as the weighted response of identical subunits shifted across the visual space while allowing for nonlinear computations to take place within each subunit. The subunits incorporate a quadratic filter sensitive to pairwise correlations in the stimulus. The weights associated with the subunits provide an estimate of invariance invariance of the neuron's response and also allow one to quantify non-invariant computations, when these weights are not uniform. The invariant subunit model provided better predictions compared to a non-invariant model with one subunit when tested on novel datasets for 103 out of 161 neurons. Analyzing the stimulus features associated with subunits, we found that they often formed quadrature pairs with similar positions, orientations, and spatial frequencies but with orthogonal spatial phases. This relationship was observed both for features that were associated with excitation of the neural response for features associated with the suppression of the neuron's responses. The excitatory pairs of subunits are positioned such that they form curved contours. The suppressive features had orientation that was approximately orthogonal to the orientation of the set of excitatory features. These results extend Hubel and Wiesel's description of complex cells to curves and provide a mechanistic explanation of how signals from the primary visual cortex are transformed within the extrastriate visual areas.

Disclosures: T.O. Sharpee: None. **R.J. Rowekamp:** None.

Nanosymposium

674. Extrastriate Cortex: Neural Coding

Location: 143A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:30 AM

Presentation Number: 674.10

Topic: D.04. Vision

Support: Intramural Research Programe NIMH

Title: A retinotopic basis for the division of category selectivity into lateral and ventral regions

Authors: *E. H. SILSON[1], A. W. Y. CHAN[1], D. J. KRAVITZ[1,2], C. I. BAKER[1];
[1]Lab-Brain & Cog, Natl. Inst. of Mental Hlth., Bethesda, MD; [2]Psychology Dept., The George Washington Univ., Washington, DC

Abstract: Extrastriate category-selective regions often come in pairs with one region on the lateral and the other on the ventral surface of human occipitotemporal cortex.. While this division is often interpreted in terms of different hierarchical processing stages, it may instead reflect underlying retinotopic biases. Neuroanatomically in macaque, the ventral and lateral surfaces receive the majority of input from early visual representations of the contralateral upper and lower field, respectively (Kravitz et al., 2013). This is consistent with the quadrant bias present within the lateral and ventral human object-selective regions (Kravitz et al., 2010). Here we focus on scene-selective regions, which are typically thought to contain large receptive fields that might limit any retinotopic biases. Further, global properties of scenes are thought to be critical for scene processing. Using population receptive field (pRF) mapping, condition rich event-related fMRI, and behavioral measures we tested visual field biases in lateral and ventral scene-selective regions (TOS and PPA, respectively). First, we estimated pRFs using both conventional checkerboards as well as scene fragments, the latter of which led to a three-fold increase in the proportion of voxels showing significant pRFs. For both stimulus types pRFs within TOS and PPA exhibited a striking contralateral bias coupled with a pronounced upper field bias in PPA and lower field bias in TOS. In contrast to object- and face-selective regions, pRFs in PPA and TOS were twice as large and exhibited a strong peripheral bias. Second, in an event-related experiment participants performed a demanding fixation task whilst images of scenes were presented randomly into one of the four quadrants of the visual field. Consistent with the pRF estimates, scene representations in PPA were strongest for the contralateral upper quadrant, whereas in TOS representations were strongest for the contralateral lower quadrant. Finally, our behavioral experiment was based on our previous observation of a prominent open/closed distinction in the responses of PPA (Kravitz et al., 2011). Consistent with an upper field bias in PPA, we observed better behavioral performance in the upper than lower visual field when making open/closed but not manmade/natural judgments. These data firmly establish that the lateral and ventral scene-selective areas, TOS and PPA, contain functionally and behaviorally relevant biases for the contralateral lower and upper visual fields, respectively. These lateral-

ventral spatial biases likely reflect a general organizing principle of the lateral and ventral portions of the ventral visual pathway.

Disclosures: **E.H. Silson:** None. **A.W.Y. Chan:** None. **D.J. Kravitz:** None. **C.I. Baker:** None.

Nanosymposium

675. The Neurobiological Sequelae of Early-Life Stress

Location: 206

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 675.01

Topic: E.05. Stress and the Brain

Support: NSF Grant IOS-1122074

Title: Early-life stress and its impact on the adolescent amygdala

Authors: ***S. L. KIGAR**[1], A. P. AUGER[2];
[1]Mol. and Cell. Pharmacol. Program, [2]Psychology, Univ. of Wisconsin-Madison, Madison, WI

Abstract: Early life experiences such as stress or immune system activation are contributing factors in the etiology of psychiatric disorders. Mechanistically, this may occur via perturbations to the epigenetic machinery involved in chromatin remodeling. Indeed, aberrant chromatin modifications are consistently observed in the postmortem tissue of psychiatric patients, and appear to be a product of dysregulated epigenetic programming during discrete periods of development. While factors involved in writing (methylating) and reading (binding methylated DNA) the epigenome, are being elucidated, less is known about the factors erasing (demethylating) the epigenome. The development and establishment of DNA methylation patterns within the neonatal amygdala are critical to the formation of typical social behavior in rodent models of psychiatric conditions. Specifically, juvenile social play behavior, a salient indicator of social and cognitive development, as well as the first non-mother directed social behavior to emerge in childhood, is sensitive to early-life perturbations of the epigenome. We find that transiently reducing the methyl-binding protein, MeCP2, within the neonatal amygdala results in a lasting reduction in juvenile social play behavior in males. Conversely, reducing the expression of the putative DNA demethylase Gadd45b within the neonatal amygdala results in a lasting increase in juvenile social play behavior in males. We also find that the transient reduction in Gadd45b results in lasting alterations in gene expression that is associated with promoter demethylation within the juvenile amygdala.. These data suggest that disturbances in the levels of proteins involved in writing, reading, or erasing the epigenome early in

development may have a causal role in affecting adverse behavioral outcomes later in life. We are now examining how early-life adversity alters the expression of these epigenetic factors involved in writing, reading, or erasing epigenetic signatures within the amygdala. We are specifically examining the amygdalae of rats exposed prenatally to LPS. These animals exhibited a significant reduction in play behavior, which may be a pertinent model for psychiatric disorders. Interestingly, males exhibit decreased mRNA levels of BDNF in the amygdala. We also saw a reduction in BDNF expression with Gadd45b knockdown, which may suggest Gadd45b is involved in stress-sensing. Furthermore, the sex-specific effect may contribute to our understanding of why males are at greater risk for neurodevelopmental disorders that affect social behavior, and further studies looking at promoter methylation are ongoing.

Disclosures: S.L. Kigar: None. **A.P. Auger:** None.

Nanosymposium

675. The Neurobiological Sequelae of Early-Life Stress

Location: 206

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 675.02

Topic: E.05. Stress and the Brain

Support: NIH Grant MH84970

Title: Stress during adolescence exerts distinct effects on excitatory drive of basolateral amygdala

Authors: *J. ROSENKRANZ, M. PADIVAL;
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Abstract: Repeated stress leads to hyperactivity of the basolateral amygdala (BLA) in adult rats. In parallel, repeated stress enhances anxiety-like behavior. However, the effects of repeated stress on BLA activity during adolescence are less clear. Excitatory drive is a key contributor to activity of BLA neurons, which in turn is associated with anxiety. These studies tested whether repeated stress during adolescence exerts distinct effects on excitatory drive of BLA neurons. Stress exposure (daily restraint, 20 minutes) was limited to postnatal days 29-39. One day after the final restraint anxiety-like behavior was measured. The following day, rats were prepared for in vivo electrophysiology or Golgi anatomical studies. During in vivo electrophysiological studies we found increased excitatory drive of BLA neurons, measured as increased responses to iontophoretically-applied glutamate and increased evoked responses to stimulation of excitatory

inputs. In Golgi-Cox stained tissue we found increased synaptic spines in subnuclei of the BLA, consistent with increased glutamatergic input to a population of BLA neurons. However, the pattern of these electrophysiological and anatomical changes in rats stressed during adolescence was different than the changes previously observed in rats stressed during adulthood. These results indicate that a distinct pattern of changes occurs in adolescent BLA after repeated stress, which may contribute to some of the differences observed in adolescent anxiety behavior after stress.

Disclosures: J. Rosenkranz: None. **M. Padival:** None.

Nanosymposium

675. The Neurobiological Sequelae of Early-Life Stress

Location: 206

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 675.03

Topic: E.05. Stress and the Brain

Support: NIGMS (1P20GM103653)

Title: Effects of caregiver maltreatment on adolescent fear-related behavior and cns dna methylation

Authors: *T. S. DOHERTY, T. L. ROTH;
Univ. of Delaware, Newark, DE

Abstract: Quality of maternal-infant interactions plays an influential role in the development of behavior and possibly in the later development of psychiatric disorders. One important mechanism of these behavioral outcomes may be environmentally-driven epigenetic changes in the central nervous system, particularly in brain regions involved in regulation of emotion and stress responsivity. One such change is DNA methylation, which results in changes in gene regulation via the addition of methyl groups to cytosines, typically resulting in transcriptional repression. Previous work from our laboratory has shown that the brain-derived neurotrophic factor (bdnf) gene, a critical player in development and synaptic plasticity, exhibits aberrant methylation in the adolescent and adult medial prefrontal cortex in response to early-life stress (brief and repeated exposures to caregiver maltreatment). We have also found methylation alterations in the adult hippocampus and amygdala following these manipulations. Our goal here was to determine whether there are aberrant methylation patterns in the adolescent hippocampus and amygdala (a time point we previously have not examined), and whether fear-related

behaviors are altered in maltreated-animals. 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. We then investigated methylation of the bdnf gene in the hippocampus (dorsal vs. ventral) and amygdala (homogenate of central, lateral, and basolateral nuclei) of these rats once they reached adolescence (PN30, at baseline conditions). Results indicate significantly higher levels of methylated DNA associated with bdnf exon IV in the ventral hippocampus and amygdala of maltreated-rats. Interestingly, these effects were found only in females. These data provide further empirical support of DNA methylation modifications as biological consequences of exposure to maltreatment. We are currently examining fear-related behaviors in these adolescent rats (both male and female) to assess the possibility of deficits in acquisition, consolidation, or extinction of conditioned fear in maltreated-rats.

Disclosures: T.S. Doherty: None. **T.L. Roth:** None.

Nanosymposium

675. The Neurobiological Sequelae of Early-Life Stress

Location: 206

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 675.04

Topic: E.05. Stress and the Brain

Support: NIH-MH091451

NIH-DC009910

Title: Influence of maternal presence on amygdala electrical activity in infancy with early life trauma

Authors: *E. C. SARRO[1,2,3,5], D. A. WILSON[2,4,6,7], R. M. SULLIVAN[2,4,5,8];
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Abstract: The survival of the infant is highly dependent on identifying, learning and approaching the maternal caregiver. This process results in the formation of an attachment to the caregiver. It is clear that the maternal caregiver can exert long lasting changes to brain function and behavior, where poor maternal care can lead to adverse consequences on adult behavior- such as an increased depressive-like phenotype. We have previously shown that infant cortical activity can be directly influenced by maternal presence and her behaviors in the nest, however it is not known whether prior early-life trauma can modulate this maternal influence on infant brain function. We addressed this initial question by implanting infant rat pups (PN12) with bipolar stainless steel electrodes, targeting the amygdaloid complex and connected to a wireless transmitter (DSI) implanted subcutaneously. Prior to implantation, one group of infants were reared from PN8-12 with a naturally abusive mother and another group were classically conditioned at PN8-12 using an odor-shock conditioning paradigm- both paradigms lead to long term behavioral deficits. We recorded spontaneous amygdala local field potential (LFP) activity daily for up to 7 consecutive days in freely behaving infants, in their natural nest environment, specifically assessing changes in LFP activity during interactions with the mother and littermates, as well during periods of specific behavioral states (i.e., nursing, grooming, milk ejections). Finally, we will briefly present data showing that the maternal odor retains important value into adulthood. We compared adult animals, with or without infant abuse and found that the odor associated with infant abuse continues to change amygdala LFP activity. Key words: maternal care, development, local field potential, amygdala, neural circuit development, telemetry

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

Nanosymposium

675. The Neurobiological Sequelae of Early-Life Stress

Location: 206

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 675.05

Topic: E.05. Stress and the Brain

Support: NIH Grant MH099910

NIH Grant MH087597

NIH Grant MH091258

Title: Dynamic sex differences during PFC maturation and their disruption by chronic stress

Authors: *A. B. RODGERS, K. E. MORRISON, T. L. BALE;
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Abstract: Environmental adversity, such as neglect or abuse, substantially increases neuropsychiatric disease risk, especially when it occurs during the years preceding puberty. Prepubertal stress experience may uniquely affect neurodevelopment in males and females, evidenced by significant sex biases in disease onset, prevalence, and symptom presentation. Maturation of the prefrontal cortex (PFC) is of particular importance, as it undergoes significant development during adolescence and plays a well-defined role in neuropsychiatric disease. In our current studies, we hypothesize that prepubertal PFC development in the mouse is sex-specific, and that exposure to environmental adversity shifts this trajectory of PFC maturation. We examine male and female mice at time points across the prepubertal window (postnatal day (PN) 21, PN28, and PN35), exposing a subset of animals to chronic variable stress. We find sex differences in gene expression in the prepubertal PFC through Affymetrix GeneChip microarray analysis and identify dynamic patterns consistent with the multi-factorial regulation of PFC maturation. We characterize the role of three factors involved in the development of sex differences in the PFC: gonadal hormones, sex chromosome complement, and epigenetic regulators. Interestingly, a novel subset of genes, including several microRNAs, displayed robust sex differences in expression at PN28 only. Sex differences in histone modifications, e.g. H3 acetylation, may underlie this sex-specific gene expression, highlighting a role for the epigenetic regulation of PFC development. Importantly, sex differences in cortical development are reflected in behavior measures of PFC function, such as the acoustic startle response, in which adolescent females display a significantly lower startle response than adolescent males. Sex differences in neurotransmitter levels in the PFC are consistent with behavioral sex differences, as HPLC analysis reveals significantly lower levels of GABA and norepinephrine in females. Notably, all observed sex differences in prepubertal PFC maturation, with the exception of chromosomally-mediated gene expression, were disrupted by chronic variable stress. These data demonstrate that prepuberty is an important window for sexual differentiation of the cortex and provide an opportunity to examine mechanisms by which prepubertal adversity results in sex biased negative outcomes, together emphasizing that neuropsychiatric disease vulnerability must be approached from the perspective of sex differences.

Disclosures: **A.B. Rodgers:** None. **K.E. Morrison:** None. **T.L. Bale:** None.

Nanosymposium

675. The Neurobiological Sequelae of Early-Life Stress

Location: 206

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 675.06

Topic: E.05. Stress and the Brain

Support: NIH grant MH41256 to BM

Title: RNA-sequencing after translating ribosomal affinity purification (TRAP) identifies *in vivo* gene expression differences in CA3 neurons of mice subjected to early life stress (ELS)

Authors: *J. D. GRAY, T. G. RUBIN, B. S. MCEWEN;
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Abstract: Exposure to early life stress (ELS) has been correlated with increased incidence of mental disorders later in life, particularly higher rates of post-traumatic stress disorder (PTSD). Several rodent models of ELS have been developed to identify the cellular and molecular changes in the brain that might be associated with increased susceptibility to mental disorders. In this study, transgenic mice expressing an EGFP fused to the L10a ribosomal subunit that is under the control of a cell-type specific promoter (Gprin3) were used to isolate the in vivo translating RNA fractions from a genetically homogenous population of CA3 neurons. Mice expressing Gprin3-EGFP-L10a were subjected to bedding and nesting deprivation from P2-P12 and then given standard housing conditions until 4mos of age. Mice were rapidly decapitated and the hippocampus was wet dissected for RNA isolation by Translating Ribosomal Affinity Purification (TRAP). TRAP immunoprecipitated and unbound fractions were subjected to RNA-sequencing using an Illumina Hi-Seq 2500 to collect 100bp reads at a sequencing depth of 30M reads/sample. Results were aligned against the mouse genome (mm9) and the numbers of reads for each transcript were normalized against total reads to obtain relative expression levels. Genespring software was used to perform statistical analysis of relative expression levels to identify differentially expressed genes. Cluster analysis demonstrated a distinct separation between bound and unbound fractions and enrichment of either neuronal or glia genes was apparent in the appropriate fraction, suggesting the TRAP was successful. Comparison of TRAP fractions between stress and control revealed over 8,120 entities changed (4,428 increased; 3,692 decreased) by ELS. Pathway analysis identified metabolic (insulin signaling, mitochondrial function) and inflammatory (TNFα and Nf-Kb signaling) gene networks as having a significant number of transcripts whose levels were changed with ELS. Splice variant and gene ontology (GO) analysis of genes differentially expressed are ongoing. As an expansion of our previous study of acute and chronic stress (Gray et al. Mol Psych 2014), these new in vivo gene expression profiles provide a refined map of stress-induced hippocampal changes that are unique to CA3 neurons and will help unravel the mechanisms underlying the unique plasticity and damage-vulnerability of these cells to ELS.

Disclosures: J.D. Gray: None. **T.G. Rubin:** None. **B.S. McEwen:** None.

Nanosymposium

675. The Neurobiological Sequelae of Early-Life Stress

Location: 206

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 675.07

Topic: E.05. Stress and the Brain

Support: MH-078105

MH-078105-S1

MH086203

OD P51OD011132

Title: Poor maternal care impacts the development of prefrontal cortex, cognitive function and social behavior in nonhuman primates: The juvenile period

Authors: *M. SANCHEZ[1], D. GUZMAN[2], B. R. HOWELL[1], T. L. VRATANINA-SMOOT[3], K. M. MCCORMACK[4], Y. SHI[5], M. STYNER[5], J. BACHEVALIER[1], D. I. SHARPE[6]; [1]Yerkes Natl. Primate Res. Ctr., [2]Emory Univ., Atlanta, GA; [3]Olivet Col., Olivet, MI; [4]Psychology, Spelman Col., Atlanta, GA; [5]Univ. of North Carolina, Chapel Hill, NC; [6]Univ. of Georgia, Athens, GA

Abstract: Child maltreatment is linked to increased risk for psychopathology, and social and cognitive deficits. This could be due to stress-induced alterations in the development of the prefrontal cortex (PFC) and its connections with amygdala and ventral striatum, critical for emotional regulation, motivation/reward, inhibitory control of behavior and executive function. How maltreatment-related alterations emerge during development is not clearly understood and difficult to study in humans. Our group utilizes a well-established and translational nonhuman primate model of maternal maltreatment (MALT) to examine these questions. In this model, maltreatment consists of two comorbid behaviors that cause infant distress: physical abuse and rejection. In addition, a unique cross-fostering experimental design allows us to study effects of experience independent of heritable factors. Recent findings using this design show increased emotional reactivity and exposure to stress hormones, as well as alterations in PFC development, in MALT animals during infancy. Here we examined the long-term effects of the adverse experience on social behavior and PFC-dependent cognitive function during the juvenile period, and their associations with neuroimaging measures of PFC structural and functional integrity. At 18 months of age, we collected measures of social behavior in 36 juvenile macaques (19 C, 17 MALT) and assessed them in a battery of PFC-related cognitive tasks: (1) Object Retrieval

Detour (ORD) task, to evaluate impulsivity and cognitive flexibility, and (2) Delayed-Non-Matching-to-Sample Session Unique (DNMS-SU) task, to evaluate working memory. MALT juveniles engaged less in social behaviors with others (affiliation, play) and also showed specific cognitive alterations. On the ORD, animals that experienced high rates of maternal rejection in infancy were more likely to stop responding following a failed attempt on a trial, suggesting lower motivation (i.e. rather than persisting in the pursuit of their goal, they quit responding). On the DNMS-SU task, animals that experienced more abuse committed more errors to criterion, suggesting impaired working memory in MALT animals, as compared to controls. Altogether, these cognitive deficits suggest potential underlying alterations in neural networks that involve the PFC and its interactions with the ventral striatum, as well as parietal cortex and hippocampus. We are currently analyzing PFC structural and functional connectivity data with these areas to test this hypothesis.

Disclosures: **M. Sanchez:** None. **D. Guzman:** None. **B.R. Howell:** None. **T.L. Vratanina-Smoot:** None. **K.M. McCormack:** None. **Y. shi:** None. **M. Styner:** None. **J. Bachevalier:** None. **D.I. Sharpe:** None.

Nanosymposium

675. The Neurobiological Sequelae of Early-Life Stress

Location: 206

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 675.08

Topic: E.05. Stress and the Brain

Support: NICHD

Title: Behavioral, biological, and epigenetic consequences of different early social experiences in rhesus macaques

Authors: S. J. SUOMI[1], *D. P. FRIEDMAN[2], A. J. BENNETT[3];
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Abstract: Objectives: This paper describes the effects of different early social experiences on behavioral, biological, and epigenetic development in rhesus monkeys. Recent research efforts designed to reverse some of these early experience effects will be also be described, as well as a strategy for documenting the efficacy of these interventions. Methods: Prospective longitudinal

studies of rhesus monkeys growing up in different early social environments (maternal vs. peer-rearing) were carried out in which both individual and rearing group differences in patterns of behavioral, biological, and epigenetic development were examined as a function of differences in early social experiences. Multiple measures of behavior, HPA axis activity, serotonin metabolism were collected longitudinally, and MRI and PET scans and longitudinal measures of genome-wide methylation and expression were also obtained. Studies of early infant imitative capabilities used both videotaped behavioral records and EEG recordings. Similar measures are being obtained in ongoing intervention studies. Results: Differences in early social rearing experiences during the first 6-7 months of postnatal life) were associated with significant differences in behavioral development, especially exploration and play, in emotional regulation, particularly fear and aggression, in neuroendocrine functioning, as reflected in HPA activity, in central serotonin metabolism, as reflected in CSF 5-HIAA concentrations, in brain structure and function, assessed via MRI and PET neuroimaging, and in genome-wide methylation pattern, both in brain tissue and in lymphocytes, and in genome-wide expression in leukocytes. Early rearing condition differences were also found in neonatal imitation capabilities, both behaviorally and in brain EEG patterns of activity. Preliminary data from intervention pilot studies indicate that many of these behavioral, biological, and epigenetic differences are reversible. Conclusions: Differences in early social experiences, particularly those involving attachment relationships, are associated with significant differences in behavioral and biological functioning, as well as in genome-wide patterns of mythelation and gene expression. It appears that social experiences during the very first days and weeks of life may have far more profound consequences for behavioral and biological development in this species than was previously suspected. Experimental efforts to “reverse” the behavioral, biological, and epigenetic consequences of these adverse early experiences are currently underway, and initial results appear promising.

Disclosures: **S.J. Suomi:** None. **D.P. Friedman:** None. **A.J. Bennett:** None.

Nanosymposium

675. The Neurobiological Sequelae of Early-Life Stress

Location: 206

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 675.09

Topic: E.05. Stress and the Brain

Support: NIH MH071537

NIH MH100122

NIH HD071982

Title: Direct and intergenerational effects of childhood trauma: Physiological, neuroimaging, and epigenetic correlates

Authors: *K. J. RESSLER[1], T. JOVANOVIC[2], J. STEVENS[2], T. KLENGEL[2], A. SMITH[2], E. BINDER[3], B. BRADLEY[2];
[1]Dept Psychiatry & Behavav Sci., Emory Univ., ATLANTA, GA; [2]Emory Univ., Atlanta, GA; [3]Max Planck Inst., Munich, Germany

Abstract: BACKGROUND: A growing number of studies indicate that low income, African American men and women living in urban environments are at high risk for trauma exposure, which may have intergenerational effects. The current study employed epigenetic, psychophysiological and neuroimaging methods to describe biomarkers of anxiety in adults with a history of childhood trauma and their at-risk children. METHODS: Study participants were recruited from a highly traumatized urban population, comprising mother-child pairs that included school-age children. Mothers were assessed for childhood abuse with the Childhood Trauma Questionnaire, the adult Traumatic Events Inventory, as with validated instruments measuring symptoms of depression and posttraumatic stress disorder (PTSD). Adults and children were measured for dark-enhanced startle responses, neuroimaging correlates of fear, and epigenetic measures of DNA methylation in peripheral blood. RESULTS: Dark-enhanced startle was found to be higher in adults with a history of PTSD and in children whose mothers had high levels of childhood physical abuse. Furthermore, we found increased amygdala activation to fearful faces as a function of trauma exposure, and differential DNA methylation in adults with a history of trauma exposure and PTSD as well as in offspring of these adults. CONCLUSION: These results demonstrate that adults with a marked history of trauma and their offspring are at higher risk for PTSD and biomarkers of risk for trauma-related disorders, including physiological, neuroimaging and peripheral blood DNA methylation. These findings emphasize the utility of physiological measures as pervasive biomarkers of psychopathology that can easily be measured across at-risk populations.

Disclosures: K.J. Ressler: None. **T. Jovanovic:** None. **J. Stevens:** None. **T. Klengel:** None. **A. Smith:** None. **E. Binder:** None. **B. Bradley:** None.

Nanosymposium

675. The Neurobiological Sequelae of Early-Life Stress

Location: 206

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 675.10

Topic: E.05. Stress and the Brain

Support: Australian Research Council (ARC; Discovery Grant DP0878136

Title: Early-life stress, adolescent brain development, and the emergence of depression

Authors: *N. B. ALLEN, S. WHITTLE, M. DENNISON, N. VIJAYAKUMAR;
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Abstract: The deleterious effects of childhood maltreatment and early life stress on brain development have been well documented. However, it is not known if and when these effects emerge during adolescence and whether comorbid adolescent emergent psychopathology is more likely to explain these effects. We will investigate whether childhood maltreatment is associated with the development of brain structure over three wave of longitudinal within subject brain scans covering early, mid- and late-adolescence (ages approximately 12, 16 and 18 years old respectively). We will evaluated whether this pattern of brain development was predicted by, or predicted the emergence of psychopathology, especially mood anxiety and conduct disorders. One hundred seventeen (60 male) adolescents, recruited as part of a broader adolescent development study, participated in magnetic resonance imaging assessments during early, mid- and late-adolescence (mean age at baseline 12.62 years, SD 0.44 years), and completed self-report measurements of childhood maltreatment, parent report of prenatal and perinatal events, and completed diagnostic interviews assessing DSM-IV mental disorders at each assessment wave. Childhood maltreatment was associated with larger baseline left hippocampal volumes and retarded growth of the left amygdala over time and was indirectly associated, through the experience of psychopathology, with retarded growth of the left hippocampus and accelerated growth of the left amygdala over time. Cortical analysis showed that maltreatment influenced thickening of the superior parietal region through the experience of psychopathology. Childhood maltreatment and early life stress was associated with altered brain development during adolescence. The experience of psychopathology during adolescence may be one mechanism by which childhood maltreatment has continuing effects on brain development during the adolescent years. These findings highlight the importance of early intervention for individuals who have experienced childhood maltreatment and other forms of early stress.

Disclosures: **N.B. Allen:** None. **S. Whittle:** None. **M. Dennison:** None. **N. Vijayakumar:** None.

Nanosymposium

675. The Neurobiological Sequelae of Early-Life Stress

Location: 206

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 675.11

Topic: E.05. Stress and the Brain

Support: NIH Grant RL1DA024856

NIH Grant R01MH69747

NIH Grant R01MH070902

NIH Grant UL1DE19586

NIH Grant T32 2MH014276

Title: Child maltreatment and corticostriatolimbic development in adolescence: Effects of maltreatment subtype and gender

Authors: *E. T. COX, F. WANG, J. JOHNSTON, L. SPENCER, C. M. MAZURE, R. SINHA, L. MAYES, H. P. BLUMBERG;
Yale Univ., New Haven, CT

Abstract: The adolescent/young adult epoch is a critical period in the development of corticostriatolimbic (CSL) neural systems that subserve impulse and emotional regulation. During adolescence, emotional symptoms and risky and addictive behaviors often emerge, with rates higher in individuals who have experienced childhood maltreatment (CM). Previous cross-sectional findings suggest CM has effects on CSL systems in adolescence, potentially contributing to these detrimental behavioral outcomes. In this longitudinal study, 44 adolescents/young adults with a history of CM (57% female) participated in two clinical and behavioral assessments, including completion of the Childhood Trauma Questionnaire (CTQ), and multimodal structural, diffusion tensor and functional imaging during emotional face processing. At initial scan the average age was 15.6 yrs, and at follow-up 18.3 yrs, with an average inter-scan interval of 2.7 yrs. The relationship between CTQ scores, including total and subscale scores, and structural and functional CSL trajectories were assessed. Findings were especially robust for white matter (WM) and included an inverse association between total CTQ scores with ventral frontal WM structural integrity over time ($p<0.05$). Physical abuse was associated with frontal, temporal, and striatal WM structural integrity decreases ($p<0.05$) over time. Gender-related associations with emotional abuse were observed. Both sexes showed

frontal WM findings, but only males showed a significant inverse association between CTQ scores with striatal WM structural integrity and females with temporopolar WM structural integrity ($p<0.05$). Functional imaging findings demonstrated effects especially for responses to fearful faces, for example, demonstrating effects especially for physical neglect in anterior cingulate cortex ($p<0.05$). Results extend previous cross-sectional CSL findings associated with CM, demonstrating their progression over adolescence. These include evidence for differing effects of CM subtypes on CSL trajectories. Gender-specific findings observed may be related to divergent CM associations in behavioral outcomes in males and females, observed previously and in this study, with highly stressed males more likely to develop externalizing behaviors and highly stressed females more likely to develop internalizing and depressive symptoms.

Disclosures: E.T. Cox: None. **F. Wang:** None. **J. Johnston:** None. **L. Spencer:** None. **C.M. Mazure:** None. **R. Sinha:** None. **L. Mayes:** None. **H.P. Blumberg:** None.

Nanosymposium

675. The Neurobiological Sequelae of Early-Life Stress

Location: 206

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 675.12

Topic: E.05. Stress and the Brain

Support: R01AA016274

R01DA033369

R01DA031579

T32HD07376

P30DA023026

Title: Decrease in reward-related ventral striatum reactivity during adolescence reflects early life stress and predicts depressive symptomatology

Authors: *J. L. HANSON[1], D. E. WILLIAMSON[3,4], A. R. HARIRI[1,2];
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[3]Psychiatry, [4]Epidemiology & Biostatistics, Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX

Abstract: Emerging evidence implicates dysfunction of reward-related neural circuitry in the pathophysiology of mood and substance use disorders, which are commonly precipitated by stressful life events. The impact of stressful life events on reward-related neural circuitry associated with psychopathology, however, is poorly understood. Here, we examined associations between early life stress (ELS), which may be particularly important in triggering later psychopathology, and the development of reward-related ventral striatum (VS) reactivity during adolescence. After a stringent, multi-level quality assurance protocol, two waves of fMRI data were available in 106 adolescents from the ongoing Teen Alcohol Outcomes Study (TAOS). Participants were scanned on a card-guessing paradigm that reliably elicits reward-related VS reactivity when they were 11-15 years old and again approximately two years later. Longitudinal analyses revealed that greater levels of ELS, specifically emotional neglect, predicted a decrease in reward-related VS reactivity from Wave 1 to Wave 2 (t=-2.1, p=.047). A decrease in VS reactivity from Wave 1 to Wave 2 also predicted greater depressive symptomology at Wave 2 (t=-2.42, p= 0.017). These results suggest that stress, particularly early in life, may contribute to the later emergence of psychopathology through decreased reward-related brain function.

Disclosures: J.L. Hanson: None. **D.E. Williamson:** None. **A.R. Hariri:** None.

Nanosymposium

675. The Neurobiological Sequelae of Early-Life Stress

Location: 206

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 675.13

Topic: E.05. Stress and the Brain

Support: NIH/NIMH Grant 1RO1MH091864

Title: Human chromosomal modification associated with early-life stress induced adolescent depression and nucleus accumbens hyporeactivity

Authors: *B. GOFF[1], L. GABARD-DURNAM[1], D. G. GEE[1], J. FLANNERY[2], D. S. LUMIAN[1], D. S. FARERI[1], C. J. CALDERA[1], N. TOTTENHAM[1];
[1]UCLA, Los Angeles, CA; [2]Univ. of Oregon, Eugene, OR

Abstract: Exposure to early-life stress (ELS) has consistently been associated with a range of negative health outcomes that include increased risk for psychopathology, particularly for disorders of emotion regulation such as depression. Emerging evidence suggests telomere erosion, a marker of biological aging, may provide insight into the mechanisms that underlie how

negative early-life experiences alter psychobiological processes at a genetic level. The current study examined the effects of ELS in previously institutionalized children and adolescents and healthy comparison youth across a wide age range (ages 3-17 years old). Consistent with previous research, the findings suggest that depression increases with the onset of adolescence in individuals with a history of ELS. Additionally, fMRI results showed atypical nucleus accumbens (NAcc) development, where the ELS group did not show a typical age-related increase in NAcc reactivity during adolescence. Consequently, the ELS group showed NAcc hypoactivation during adolescence, and lower NAcc reactivity was associated with higher depression scores. Findings also demonstrated that individuals with a history of ELS have significantly shorter telomere length than the comparison group and this disparity is greatest in early childhood. A negative correlation between depression scores and telomere length was also observed. The results from the current study have important implications for understanding the associations between ELS exposure, depression, and genetic changes and may represent a significant advancement for the study of deleterious psychological outcomes in this population.

Disclosures: B. Goff: None. **L. Gabard-Durnam:** None. **D.G. Gee:** None. **J. Flannery:** None. **D.S. Lumian:** None. **D.S. Fareri:** None. **C.J. Caldera:** None. **N. Tottenham:** None.

Nanosymposium

676. Biological Rhythms and Sleep: Mechanisms

Location: 152B

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 676.01

Topic: E.08. Biological Rhythms and Sleep

Support: NSF DGE-1144086

NIH R01 NS072431

Title: Regulation of the sleep/wake cycle in *Drosophila* by dADAR and its effectors

Authors: *J. E. ROBINSON, M. WU, W. J. JOINER;
Pharmacol., Univ. of California San Diego, La Jolla, CA

Abstract: Sleep is an essential, homeostatic process that is highly conserved throughout the animal kingdom. Despite being intensely studied, the molecular events that control sleep are still poorly understood. Here we use Drosophila melanogaster as a model organism to study the genetic basis of sleep regulation. Using transgenic RNAi's to knock down different genes in the

nervous system, we have identified the RNA editing gene, dAdar, as essential for normal sleep. We show that reduction in dAdar expression increases sleep by reducing the stability of the wake state. We further show that this phenotype can be reversed by selective knockdown of two edited targets of dADAR. Interestingly, these targets comprise a single conserved signaling complex whose upstream activator is upregulated when dAdar levels are reduced in the brain. Thus, under wild-type conditions, dAdar downregulates a specific signaling pathway in the brain to stabilize the wake state. Although coordinate RNA editing of thousands of transcripts is believed to keep neuronal function within a tight physiological range, our results point to a specific role for editing of functionally related transcripts in regulating homeostatically controlled processes such as the sleep/wake cycle.

Disclosures: J.E. Robinson: None. **W.J. Joiner:** None. **M. Wu:** None.

Nanosymposium

676. Biological Rhythms and Sleep: Mechanisms

Location: 152B

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 676.02

Topic: E.08. Biological Rhythms and Sleep

Title: Brain temperature and wide-band electrical brain activity in a zebra finch during sleep

Authors: *A. L. VYSSOTSKI, A. E. STEPIEN;
Inst. of Neuroinformatics, Univ. of Zurich and ETH Zurich, Zurich, Switzerland

Abstract: Multiple mammalian studies and avian studies have shown that slow-wave sleep (SWS), which comprises the largest portion of resting time in mammals, is important for the processes of memory consolidation and brain plasticity. Based on the mammalian research, the slow-wave activity (SWA) has been taken as an index of the intensity of sleep. But is SWA really the best indicator of nocturnal brain activity in birds? And how do oscillations of higher frequencies behave during the night? Recordings of electrocorticograms (ECoGs) in awake birds have shown that changes in the intensity of middle- and high-frequency electrical brain oscillations (>20 Hz) better reflect the severity of sensory stimulation and ensuing processing of this stimulus than does low-frequency brain activity. This finding motivated the hypothesis that high-frequency oscillations may therefore be a better measure of functional/cellular brain activity in sleep as well. To test this hypothesis we placed epidural AgCl recording electrodes (5-25 kOhm) over forebrain and recorded ECoGs in sleeping zebra finches in the frequency range 1 Hz - 10 kHz. Differential pairs of electrodes were placed over the right and the left hemispheres

symmetrically (AP-5.5, ML+/-1.5; AP0.0, ML+/-2.1). In addition, to measure brain temperature, microminiature thermistors have been implanted epidurally in the proximity of the right posterior electrode. Recordings were done in sound-proof metallic chamber shielding electromagnetic disturbances. We have found that oscillations in the wide frequency range 1-3000 Hz undergo changes during a 12h dark period. High-frequency activity (HFA, 0.3-3.0 kHz) associated with neuronal firing is increased at the beginning of the dark period, reaches the minimum 2 h after the light offset, and slowly increases towards the end of the dark period. Brain temperature changes co-directionally with the HFA, indicating, as expected, that HFA is associated with increased metabolic activity. SWA dynamics are opposite to those of HFA. We found that HFA increases rapidly during short episodes of REM sleep. Frequency and duration of these episodes increase towards the end of the dark period. These changes were observed in both hemispheres symmetrically. In many cases rapid increases in HFA occurred conjunctively with rising brain temperature. Notably, the changes in high- and low-frequency bands were similar in magnitude (about +/-50% from the average of the night). Our findings agree with the concept that SWA occurs in the gaps of prolonged neuronal hyperpolarizations and firing. We conclude that high-frequency oscillations provide important additional information for description of sleep.

Disclosures: A.L. Vyssotski: None. **A.E. Stepien:** None.

Nanosymposium

676. Biological Rhythms and Sleep: Mechanisms

Location: 152B

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 676.03

Topic: E.08. Biological Rhythms and Sleep

Support: Brain Science Institute Grant #80025957 to the Blackshaw and Hattar Labs

NIMH Grant #63104 to the Herzog Lab

Visual Neuroscience Training Program Fellowship to Joseph Bedont

NSF Graduate Research Fellowship to Joseph Bedont

Title: Developmental defects in the Lhx1-deficient SCN give insight into circadian physiology

Authors: *J. L. BEDONT[1], T. LEGATES[2], E. SLAT[3], M. BYERLY[1], G. WONG[1], E. HERZOG[3], S. HATTAR[2], S. BLACKSHAW[1];

[1]Neurosci., Johns Hopkins Med. Inst., Baltimore, MD; [2]Johns Hopkins Univ., Baltimore, MD; [3]Washington Univ. in St. Louis, St. Louis, MO

Abstract: Light-entrained circadian rhythms are organized by the hypothalamic suprachiasmatic nucleus (SCN) in vertebrates. Despite this important role, Lim homeodomain transcription factor 1 (Lhx1) is the only known regulator of SCN terminal differentiation. At SFN 2013, we showed that deletion of Lhx1 in the anterior hypothalamus down-regulates key SCN neuropeptides and disrupts SCN clock gene and organismal wheel-running rhythms. Attempts to restore rhythms in our mutants by icv cannulation of down-regulated neuropeptides produced seemingly paradoxical results, highlighting the remarkable interdependence of neuropeptide signaling in the SCN network. Building upon these studies, we have now identified additional down-regulated SCN-enriched genes and noted reduced responsiveness to light in Lhx1-deficient SCN; furthermore, rhythms of general activity, body temperature, and sleep are all profoundly disrupted in these mutants. We also observed a light cycle dependent increase in NREM sleep at the expense of waking in these animals. Finally, we found that the mutant circadian system is hypersensitive to non-photic entrainment cues, mirroring its hypersensitivity to icv delivery of SCN neuropeptides. These studies offer exciting new insights into SCN physiology, including possible mechanisms coupling circadian and homeostatic control of sleep, and further understanding of how the SCN copes with common stimuli disruptive to entrainment.

Disclosures: J.L. Bedont: None. **T. LeGates:** None. **M. Byerly:** None. **G. Wong:** None. **S. Hattar:** None. **S. Blackshaw:** None. **E. Slat:** None. **E. Herzog:** None.

Nanosymposium

676. Biological Rhythms and Sleep: Mechanisms

Location: 152B

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 676.04

Topic: E.08. Biological Rhythms and Sleep

Support: BBSRC Grant BB/J003441

BBSRC Grant BB/L007665

Wellcome Trust Grant 092319MA

University of Manchester Neuroscience Research Institute Studentship

Title: Feedback of locomotor activity to the suprachiasmatic nuclei and neural circadian system

Authors: *A. T. HUGHES, R. E. SAMUELS, M. D. C. BELLE, S. WEGNER, H. D. PIGGINS;
Fac. of Life Sci., Univ. of Manchester, Manchester, United Kingdom

Abstract: The daily timing of all aspects of physiology and behavior is controlled by a dominant circadian pacemaker in the suprachiasmatic nuclei (SCN) as well as local tissue-specific clocks throughout the brain and periphery. Endogenous rhythms are generated through the expression of core clock genes, including the *per* and *cry* genes, and must be coordinated with the external environment via the actions of exogenous time cues and feedback from endogenous clock-controlled outputs. Daily scheduled exercise is one such synchronizing influence and it is well established that regularly scheduled locomotor activity entrains behavioral rhythms in wild-type mice. Vasoactive intestinal polypeptide (VIP) and its receptor, $VPAC_2$, play key roles in rhythm generation and maintenance in the SCN such that mice lacking VIP ($Vip^{-/-}$) or $VPAC_2$ receptors ($Vipr2^{-/-}$) generate only low amplitude, desynchronized rhythms in SCN core clock gene expression and neuronal activity. We recently showed that placing $Vipr2^{-/-}$ mice on a regimen of daily scheduled voluntary exercise (SVE) promoted free-running behavioral rhythmicity but that only a reduced proportion of $Vip^{-/-}$ mice responded in a similar manner. We therefore generated mice lacking both VIP and the $VPAC_2$ receptor ($Vip^{-/-}$ x $Vipr2^{-/-}$) and investigated whether the loss of both elements of this signaling system rendered mice unable to respond the SVE. We demonstrate that neither VIP signaling though another receptor, nor an alternative ligand activating $VPAC_2$, are required for the beneficial effects of SVE as near 24h behavioral rhythms are effectively promoted in $Vip^{-/-}$ x $Vipr2^{-/-}$ mice. At present, it is unclear whether SVE-promotion of behavioral rhythms relies on expression of the canonical molecular clockwork. To investigate this, we subjected behaviorally arrhythmic mice lacking functional expression of the *cry1* and *cry2* core clock genes to our SVE regimen and found that SVE failed to induce behavioral rhythms in these mice. Further, as it is currently unknown whether $Vipr2^{-/-}$ SCN function is restored by SVE, we used $Vipr2^{-/-}$ mice expressing reporters of *per1* and PER2 to demonstrate that while SVE fails to alter rhythmic clock gene expression in extra-SCN hypothalamic nuclei, it significantly increases the number and synchrony of rhythmic neurons in the $Vipr2^{-/-}$ SCN. Finally, to interrogate the mechanisms behind this effect, we are assessing whether alterations in GABAergic signaling contribute to exercise-mediated enhancement of SCN rhythms in $Vipr2^{-/-}$ mice. We conclude that SVE promotes behavioral rhythmicity through partial restoration of SCN function in $Vipr2^{-/-}$ mice.

Disclosures: A.T. Hughes: None. **R.E. Samuels:** None. **M.D.C. Belle:** None. **S. Wegner:** None. **H.D. Piggins:** None.

Nanosymposium

676. Biological Rhythms and Sleep: Mechanisms

Location: 152B

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 676.05

Topic: E.08. Biological Rhythms and Sleep

Support: CHDI Grant A-7293

Title: The Q175 mouse model of Huntington's disease shows decline in circadian rhythms of SCN electrical activity

Authors: *T. KUDO, C. COLWELL;
UCLA, Los Angeles, CA

Abstract: Many patients with Huntington's disease (HD) exhibit disturbances in their daily cycle of sleep and wake as part of their symptoms. These patients have difficulty sleeping at night and staying awake during the day, which has a profound impact on the quality of life of the patients and their care-takers. For developing treatments of the human disease, knock-in (KI) models offer advantages of genetic precision and control of mutation copy number. Therefore, we used a relatively new model of HD with an expansion of the KI repeats (Q175). In the previous paper, we found Q175 showed circadian disruption in the behavior, but the SCN electric activity is not yet examined. Hence, we examined the SCN electric activity in pre-symptomatic (5 months of age) and symptomatic (7 months of age) Q175 mouse. In pre-symptomatic stage, day-night difference was lost in Q175 mouse, but there was no difference with wild-type mouse during day time. In symptomatic stage, day-night difference was lost and day time firing rates was significantly reduced in Q175 mouse during day time. Together, this data is consistent with the hypothesis that the HD mutations interfere with the expression of robust circadian rhythms in behavior and physiology. The data raise the possibility that the electrical activity within the central clock itself may be altered in this disease.

Disclosures: T. Kudo: None. **C. Colwell:** None.

Nanosymposium

676. Biological Rhythms and Sleep: Mechanisms

Location: 152B

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 676.06

Topic: E.08. Biological Rhythms and Sleep

Support: Davis Postdoctoral Fellowship

HHMI

Title: Preoptic hypothalamus modulation of sleep

Authors: *S. CHUNG[1], F. WEBER[1], W.-C. CHANG[1], A. CETIN[2], Y. DAN[1];
[1]UC Berkeley, Berkeley, CA; [2]Allen Inst. for Brain Sci., Seattle, WA

Abstract: The preoptic hypothalamus (POA) plays a critical role in modulating sleep and wake states. Neurons in the POA exhibit various discharge profiles in relation to different brain states, and it has been known that the POA contains sleep active neurons and they are connected to major sleep/wake regulatory regions. First to identify whether the POA interacts with major wake promoting centers of the brain, trans-synaptic retrograde tracing was performed using pseudotyped rabies virus. The results showed that the GABAergic POA neurons monosynaptically innervate to the histamine producing neurons in the tuberomammillary nucleus (TMN). In order to identify sleep active neurons, we performed projection target specific recording of neurons in the POA. To label GABAergic neurons that project to the TMN region, rabies glycoprotein-pseudotyped lentivirus that contains channelrhodopsin-2 (ChR2)-YFP and can travel retrogradely in a cre-dependent manner was injected into the TMN of GAD2-cre mice. These retrogradely labeled cell bodies in the POA were optogenetically stimulated and its effect on sleep and wake states was measured. Furthermore, these neurons were optogenetically identified and recorded using optetrode in freely moving mice. The results of our study will increase our understanding on how selective modulation of projection target specific GABAergic neurons in the POA can induce brain states transition by interacting with the major sleep/wake regulatory brain region.

Disclosures: S. Chung: None. **F. Weber:** None. **W. Chang:** None. **A. Cetin:** None. **Y. Dan:** None.

Nanosymposium

676. Biological Rhythms and Sleep: Mechanisms

Location: 152B

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 676.07

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant NS048476

Title: Gaba excites some MCH neurons and inhibits others

Authors: A. ZAYACHKIVSKY, *D. J. SPERGEL, A. N. VAN DEN POL;
Dept Neurosurg., Yale Univ. Sch. Med., New Haven, CT

Abstract: GABA is the primary inhibitory transmitter in the adult mammalian brain, but it can also excite. The main mechanism underlying the inhibition (decrease in spike frequency) is based on inward movement of Cl^- resulting in membrane hyperpolarization due to the Cl^- reversal potential (E_{Cl}) being more negative than the resting membrane potential (RMP). The main mechanism underlying the excitation (increase in spike frequency) is based on outward movement of Cl^- resulting in membrane depolarization due to E_{Cl} being less negative than the RMP. Melanin-concentrating hormone (MCH) neurons play a critical role in energy homeostasis (food intake and body weight regulation) and sleep. MCH cells receive strong GABA synaptic input and relatively less glutamate excitatory input. We tested whether GABA inhibits or excites mature (40-60 days old) MCH neurons in MCH-GFP transgenic mouse brain slices perfused with HCO_3^--buffered artificial cerebrospinal fluid (ACSF) and recorded with the gramicidin-perforated patch technique. GABA (100 μM), by itself or when paired with depolarizing current injection to simulate glutamatergic or other excitatory input, hyperpolarized and inhibited 27% of MCH neurons, depolarized (without increasing spike frequency) 44% of MCH neurons, and excited 9% (by itself) or 29% (when paired with sub-threshold depolarizing current injection) of MCH neurons (n = 34). The $GABA_A$ agonist muscimol (10-30 μM) mimicked the effect of GABA, suggesting involvement of the $GABA_A$ receptor. The $GABA_A$ antagonist bicuculline (30-100 μM), which blocked the GABA and muscimol responses, had the opposite effect. To address the mechanism of the excitation in MCH neurons, we measured the GABA reversal potential (E_{GABA}) as well as the RMP. E_{GABA} was -52.3 mV (n = 17) in MCH neurons depolarized by GABA, and was significantly different ($p < 0.05$, unpaired t-test) than in MCH neurons hyperpolarized by GABA (-75.3 mV; n = 9). RMP was -68.3 mV (n = 20) in MCH neurons depolarized by GABA and was not significantly different ($p > 0.05$, unpaired t-test) than in MCH neurons hyperpolarized by GABA (-62.4 mV; n = 10). We also measured E_{GABA} and the RMP of MCH neurons in HEPES-buffered ACSF. Under those conditions, E_{GABA} (= E_{Cl}) was positive (-46.2 mV) to the RMP (-63.6 mV; n = 5), indicating that intracellular Cl^- concentration in MCH neurons depolarized by GABA is approximately 24 mM (calculated from the Nernst equation). Thus, GABA excites a subpopulation of mature MCH neurons with relatively high intracellular Cl^-, but inhibits others with less intracellular Cl^-.

Disclosures: A. Zayachkivsky: None. **D.J. Spergel:** None. **A.N. van den Pol:** None.

Nanosymposium

676. Biological Rhythms and Sleep: Mechanisms

Location: 152B

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 676.08

Topic: E.08. Biological Rhythms and Sleep

Support: Howard Hughes Medical Institute

Title: Imaging sleep-wake activity in identified pontine cell populations

Authors: *J. M. COX[1], L. PINTO[2], Y. DAN[3,4];
[1]Vision Sci. Grad. Program, [2]Helen Wills Neurosci. Inst., [3]Dept. of Mol. and Cell Biol., UC, Berkeley, Berkeley, CA; [4]Howard Hughes Med. Inst., Berkeley, CA

Abstract: An animal's brain state transitions rapidly and repeatedly over the course of a day, corresponding with changes in global patterns of neural activity. Decades of research have identified distinct brain regions responsible for the induction and maintenance of sleep and wake states, but it has been difficult to determine the precise role of specific neuronal subtypes within these regions. Multiple nuclei within the mesopontine tegmentum in the brainstem are important for the induction and maintenance of both rapid eye movement (REM) sleep and wakefulness. For instance, neurons in the laterodorsal tegmental nucleus (LDT) are most active during periods of wake and REM sleep, but this nucleus is comprised of multiple, intermingled cell types. In order to characterize the brain state-specific activity of identified neurons in this region, we transfected GABAergic or glutamatergic neurons located near the cholinergic neurons of the LDT, with the calcium indicator GCaMP6s. We then imaged their activity using a microendoscope and head mountable fluorescence microscope in the feely moving mouse as it transitioned naturally through the sleep wake cycle. This technique allowed us to monitor the activity of multiple cells simultaneously and define their state-specific activity patterns, indicating distinct roles in the modulation of brain state for different cell types within the LDT.

Disclosures: J.M. Cox: None. **L. Pinto:** None. **Y. Dan:** None.

Nanosymposium

676. Biological Rhythms and Sleep: Mechanisms

Location: 152B

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 676.09

Topic: E.08. Biological Rhythms and Sleep

Support: Grant-in-Aid for JSPS Fellows

Title: Characterizing complexity of intracortical interaction

Authors: *S. TAJIMA, T. TOYOIZUMI;
Brain Sci. Inst., RIKEN, Saitama, Japan

Abstract: Inter-areal interaction within brain is suggested as a critical factor for many cognitive functions. The strength of interaction has been measured by functional connectivity, such as correlation or the transfer entropy. However, only limited attempts have been made to characterize the complexity of interaction, i.e., the roll of interaction in producing dynamics of the system. Here, we propose a model-free methodology for simultaneously quantifying the strength and complexity of interactions. Embedding theorems in dynamical system claim that we can reconstruct the attractor topology for the “upstream-node” dynamics if we map the “downstream-node” dynamics into a delay-coordinate state-space with a sufficient number of dimensions. As a practical measure of embeddedness, we utilized nonlinear forecasting performance (Sugihara et al., 2012). In particular, the interaction strength is measured by how accurately a history of one node can predict the dynamics of another node, while the interaction complexity is measured by how long history of one node is necessary for the optimal prediction of another. Because this method assumes no specific model, it enables objective characterization of interactions. We applied this embedding analysis to monkey electrocorticogram (ECoG) recording from the entire cortical surface. We found (1) clear asymmetric intracortical interactions converging from the occipital to the frontal lobe, (2) high complexity interactions that are localized within the frontal areas, and (3) disappearance of those complex interactions after the state transition from awake to anesthetized conditions. Interestingly, the converging interaction and complexity were significantly reduced in specific areas including fronto-parietal cortex but not in the visual cortex. These results indicate that the fronto-parietal cortex integrates brain-wide information and embeds it within the complex dynamics. While integrative interaction and complexity have been conceptually proposed as bases of conscious experiences (Tononi & Edelman, 1998), the present results provide a structural correlate of consciousness that reflects those two aspects of brain dynamics. Hence, our promising results suggest that the present approach will be a generally applicable strategy for investigating complex interaction underlying a variety of neurophysiological network dynamics.

Disclosures: **S. Tajima:** None. **T. Toyoizumi:** None.

Nanosymposium

676. Biological Rhythms and Sleep: Mechanisms

Location: 152B

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 676.10

Topic: E.08. Biological Rhythms and Sleep

Support: NIH T32 MH019986-16, PI: CF Reynolds

MH024653, PI: D.J. Buysse, MD

5 R01 MH061566, PI: G.J. Siegle, PhD

W81XWH-08-1-0637, PI: A. Germain, PhD

Title: Slow-wave activity during non-REM sleep is associated with cerebral metabolic rate during sleep and wakefulness

Authors: *K. A. WILCKENS, E. A. NOFZINGER, J. A. JAMES, A. GERMAIN, H. J. AIZENSTEIN, G. J. SIEGLE, D. J. BUYSSE;
Dept. of Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Slow-wave activity (SWA) (0.5-4 Hz) during non-rapid eye-movement (NREM) sleep declines with aging, and is thought to be a marker for cortical reorganization, particularly within the frontal lobes. Thus greater SWA may promote cerebral metabolic rate during both sleep and wakefulness. We examined the association between absolute and relative measures of NREM slow-wave activity (aSWA and rSWA) with whole brain and regional glucose metabolism during NREM sleep and wakefulness in young and middle-aged adults. Participants included 54 young and middle-aged adults between the ages of 24-60 (mean age =38.95; 28 females). Participants were enrolled in studies that included polysomnography, quantitative EEG, and FDG positron emission tomography scans. Relative and absolute estimations of glucose metabolism were collected. Multiple regressions revealed that whole brain absolute glucose metabolism during wakefulness was marginally and positively associated with greater rSWA, R squared =0.07, p=0.076. This effect was moderated by age (R squared change=0.114, p=0.014); using a median split, the relationship was significant in older (mean age = 47.40), r=0.53, p=0.008, but not younger (mean age = 30.50) participants. There was a significant positive relationship between rSWA and metabolism during wakefulness in the right superior frontal gyrus (MNI coordinates = 8, 28, 52), t=3.86, p=0.025. Consistent with the view that SWA reflects lower frontal lobe metabolism during sleep, NREM glucose metabolism in the inferior frontal gyrus (MNI coordinates = -42, 22, 0) was negatively associated with aSWA, t=6.49, cluster level FWE

corrected, $p<0.001$. Neither of the regional metabolism effects was moderated by age. Results suggest that greater SWA is associated with greater metabolic rate during wakefulness and lower metabolic rate during NREM sleep in brain regions important for executive control. Broadly, these results suggest that greater SWA may moderate age-related changes in cerebral metabolic rate.

Disclosures: **K.A. Wilckens:** None. **E.A. Nofzinger:** None. **J.A. James:** None. **A. Germain:** None. **H.J. Aizenstein:** None. **G.J. Siegle:** None. **D.J. Buysse:** None.

Nanosymposium

676. Biological Rhythms and Sleep: Mechanisms

Location: 152B

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 676.11

Topic: E.08. Biological Rhythms and Sleep

Support: Netherlands Organization for Scientific Research (NWO) grants: VICI 453.07.001 to E.J.W.V.S.

BIAL Foundation 220/12 to G.P.

Title: Involvement of phasic brainstem activity in the preferential anterior-to-posterior direction of traveling cortical slow waves in human sleep EEG

Authors: E. J. W. VAN SOMEREN[1], G. PIANTONI[1,4], *D. STOFFERS[2], Y. D. VAN DER WERF[3], T. DANG-VU[5], P. MAQUET[6];
[1]Sleep & Cognition, [2]Psychology, [3]Emotion & Cognition, Netherlands Inst. For Neurosci., Amsterdam, Netherlands; [4]Massachusetts Gen. Hosp., Boston, MA; [5]Exercise Sci. and Ctr. for Studies in Behavioral Neurobio., Concordia Univ., Montreal, QC, Canada; [6]Neurol., Univ. of Liège, Liège, Belgium

Abstract: The slow waves of sleep arise from the alternation of periods of relative neuronal silence and periods of high neuronal activity. Intriguingly, slow waves travel preferentially from anterior to posterior cortical regions. It has been proposed that phasic activity of subcortical regions that modulate cortical excitability may be involved in state transitions. The neuronal mechanisms contributing to the directional preference of traveling however remain elusive. Since the active phase of the cortical slow wave can be preceded by phasic activity in the locus coeruleus (LC), we hypothesized that the LC, due to the anteroposterior gradient of the length

and consequently axonal propagation duration of its cortically projecting fibers, might depolarize frontal cortical neurons slightly earlier than more posterior neurons and thus prime the cortex for a preferential propagation of slow waves along the anterior-to-posterior axis. High-density electroencephalography (EEG) and functional Magnetic Resonance Imaging (fMRI) were recorded in 14 participants while sleeping. Slow waves were automatically detected and classified according to their direction of propagation on the scalp as traveling either in the anterior-to-posterior or posterior-to-anterior direction. Statistical parameter mapping (SPM) analysis was used to evaluate to which extent these two event types were preceded by phasic activation in the brainstem. A brainstem area compatible with the right LC was significantly activated in association with slow waves traveling in the anterior-to-posterior direction, but not significantly with slow waves traveling in the opposite, posterior-to-anterior, direction. Phasic activity in the LC may thus contribute to the bias of slow waves to travel in an anterior-to-posterior direction.

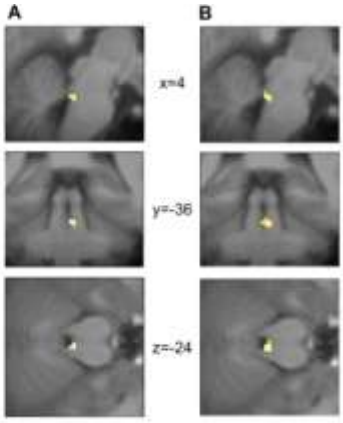

Figure: Brainstem BOLD activity with anterior-to-posterior traveling slow waves, with (A) and without (B) correction for multiple comparisons. The activation map is superimposed onto a study-specific template structural MRI, obtained by averaging the images after realignment in MNI space. The low degree of blurriness indicates that the realignment was satisfactory. (A) At the threshold of p-value 0.05, corrected for multiple comparisons, 4 voxels were significantly activated in association with anterior-to-posterior slow waves. The area showing a significant fMRI activation is compatible with the location of the right locus coeruleus (LC) in humans. (B) Even at the more liberal threshold of uncorrected p-value 0.001, the activation is limited to the pontine area compatible with the location of the LC.

Disclosures: E.J.W. Van Someren: None. **G. Piantoni:** None. **D. Stoffers:** None. **Y.D. Van Der Werf:** None. **T. Dang-Vu:** None. **P. Maquet:** None.

Nanosymposium

676. Biological Rhythms and Sleep: Mechanisms

Location: 152B

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 676.12

Topic: E.08. Biological Rhythms and Sleep

Support: Netherlands Organization of Scientific Research (NWO) VICI 4530700

Title: Brain structure and large-scale psychometrics point to different subtypes of insomnia

Authors: *E. J. VAN SOMEREN[1,2], J. BENJAMINS[1], D. STOFFERS[1], T. HOEKSTRA[1,3], F. MIGLIORATI[1], M.-J. VAN TOL[4], J. W. R. TWISK[3];
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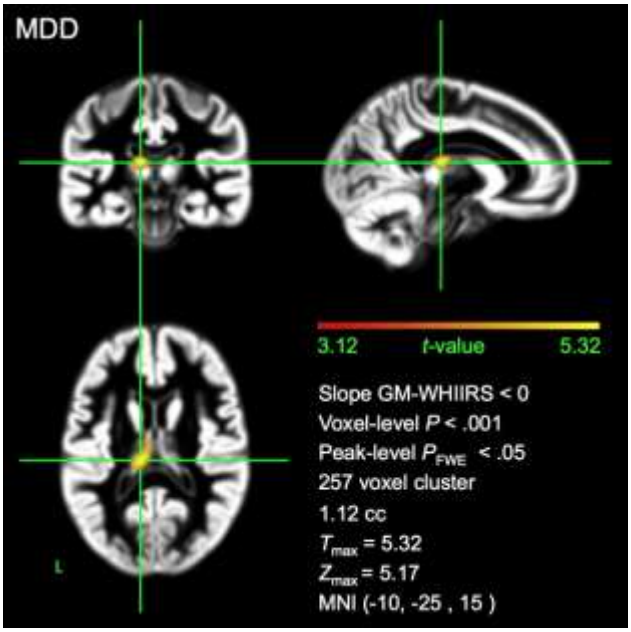
Abstract: Insomnia is a prevalent severe disorder and a major risk factor for other disorders. Unfortunately, findings on its neuronal correlates have been scarce and equivocal, hampering the development of mechanistic models and rational intervention. A possible reason for the equivocal findings is that insomnia is not a single disorder, but comes in many subtypes with different underlying causes – just like 'dementia of old age' is now recognized to involve many different pathologies that can occur both individually and concurrently. We addressed the multiple subtype hypothesis through (1) large-scale multivariate internet assessment of stable characteristics, life events and health history followed by Latent Class Analysis (LCA), as well as through (2) structural magnetic resonance imaging of carefully selected subtypes followed by whole brain Voxel Based Morphometry (VBM).LCA on 47 constructs measured by questionnaires in 1538 insomniac participants of the Netherlands Sleep Registry (www.sleepregistry.org) revealed four distinct insomnia subtypes, differing with respect to depression, anxiety, perfectionism, rumination and pain, among others. Interestingly, no subtype was distinguished by sleep constructs.VBM on 59 participants with major depressive disorder (MDD), 61 with anxiety disorder without and 78 with comorbid depression, and 62 controls revealed exclusively in MDD a strong negative association of insomnia severity with gray matter density in a large cluster in the left thalamus encompassing the entire pulvinar, independent of depression severity or use of antidepressants. Concertedly, the findings support the hypothesis that multiple subtypes of insomnia exist. If not recognized accordingly, samples are likely to be too heterogeneous to find consistent psychometric characteristics and neural correlates of insomnias. *Figure: Slices through the left pulvinar peak voxel for negative associations of gray matter probability with insomnia severity (Women's Health Initiative Insomnia Rating Scale) exclusively in Major Depression Disorder*



Disclosures: **J. Benjamins:** None. **E.J. Van Someren:** None. **D. Stoffers:** None. **T. Hoekstra:** None. **F. Migliorati:** None. **M. Van Tol:** None. **J.W.R. Twisk:** None.

Nanosymposium

676. Biological Rhythms and Sleep: Mechanisms

Location: 152B

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 676.13

Topic: E.08. Biological Rhythms and Sleep

Title: Losing control: The neural basis of affective regulation without sleep

Authors: *E. B. SIMON[1,2], H. SHARON[1,3], N. OREN[1,3], A. KIRSCHNER[3], N. GOLDWAY[3], T. HENDLER[1,4];
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Abstract: Introduction Being able to effectively regulate emotions is a key aspect of healthy functioning, crucial in tackling both cognitive and affective challenges. While sleep deprivation (SD) is known to impair cognitive function its impact on affective processing is surprisingly scarce. Using EEG and fMRI we aimed to elucidate the effects of SD on affective regulation and its underlying neural basis. Methods Eighteen healthy volunteers performed two different

cognitively demanding tasks involving emotional distractors after a full night's sleep, and again following 24-hours of total SD (in a counter-balanced order). In the first task, participants underwent an fMRI scan while performing an emotional version of the N-back task, in which affective and neutral distractors were presented in the background of a series of numbers to be remembered as either 1back or 2back. The second task probed EEG amplitudes of the well-described steady state visually-evoked potentials (SSVEP) during a demanding high load visual task that was presented with background affective distractors. Results As expected, accuracy scores declined following SD in both tasks, with worse performance observed during higher cognitive loads. N-back fMRI results revealed a significant sleep X load interaction in left amygdala activity: following sleep, left amygdala activity was inversely correlated with cognitive load, i.e. its activity decreased with increasing load. Such modulation was absent following SD, suggesting impaired capacity for affective regulation without sleep. Psycho-physiological interaction analysis with the same amygdala ROI further revealed increased load-sensitive connectivity with dorso-medial PFC only following sleep, implying a significant disruption of PFC regulation after SD. In addition, this change in PFC-amygdala connectivity was significantly correlated with deteriorated N-back accuracy scores following SD. Similarly, SSVEP amplitudes were modulated by affective distractors only following sleep, but not following SD, suggesting that impaired affective regulation can also be manifested during primary visual processing. Conclusions Our findings demonstrate that SD significantly disrupts affective regulation leading to impaired performance during cognitive-emotional interactions, as evident using both EEG and fMRI. It seems that adequate sleep is a crucial determinant in our ability to handle affective distractors. Since many of our daily activities nowadays integrate cognitive and affective challenges, it seems that a good night's sleep is more vital than ever.

Disclosures: **E.B. Simon:** None. **H. Sharon:** None. **N. Oren:** None. **A. Kirschner:** None. **N. Goldway:** None. **T. Hendler:** None.

Nanosymposium

677. Blood Brain Barrier: Development and Disease

Location: 147A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:15 AM

Presentation Number: 677.01

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R01NS065089

NIH Grant P01NS055104

NIH Grant K08NS057339

Title: Transforming growth factor-β induces matrix metalloproteinase-2 and -9 activation in human brain pericyte

Authors: *Y. TAKAHASHI[1,3,2], T. MAKI[3], A. C. LIANG[3], K. ITOH[3], J. LOK[3], N. OSUMI[2], K. ARAI[3];
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Abstract: Background: Pericytes are vascular mural cells embedded within the basement membrane of blood micro-vessels. Within the neurovascular unit, pericytes play important roles in regulating neurovascular homeostasis by secreting soluble factors, such as matrix metalloproteinases (MMPs). However, little is known about the regulatory signaling pathways in brain pericytes. Here we show that transforming growth factor-β (TGF-β) induces both MMP-2 and MMP-9 via TGF-β receptor – mitogen-activated protein kinase (MAPK) signaling. **Methods and Results:** Cultured human brain vascular pericytes were obtained from Science Cell Research Laboratories. These pericytes expressed a pericyte marker platelet-derived growth factor (PDGF)-receptor , but not an astrocyte marker (glial fibrillary acidic protein), an endothelial marker (CD31), nor an oligodendrocyte precursor cell marker (PDGF-receptor). When the brain pericytes were treated with purified human TGF-β1 (0.1- 10 ng/mL for 24 hours), the levels of MMP-2 and MMP-9 in culture media were significantly increased in a concentration dependent manner as measured by gelatin zymography. Water soluble tetrazolium salt (WST) assay confirmed that TGF-β1 did not affect cell survival of the brain pericytes. Immunostaining showed that the brain pericytes expressed TGF-β-receptor, and a TGF-β-receptor inhibitor SB431542 (0.5 - 5 M) decreased the TGF-β1-induced upregulation of MMP-2 and MMP-9. To assess the underlying intracellular mechanisms, we focused on p38 MAPK signaling, one of the major pathways for TGF-β signaling. Western blotting showed that TGF-β1 treatment increased the level of p38 MAPK phosphorylation, and again, SB431542 (0.5 - 5 M) blocked the TGF-β1-induced phosphorylation of p38 MAPK. Importantly, a p38 MAPK inhibitor SB203580 (0.5 - 5 M) cancelled the effect of TGF-β1 in upregulation of MMP-9 but not of MMP-2. **Conclusion:** These data demonstrate that in brain pericytes, (i) p38 MAPK plays an essential role in TGF-β-induced MMP-9 upregulation, and (ii) MMP-2 and MMP-9 upregulations by TGF- β are mediated by different signaling pathways. Both TGF-β and MMP-9 are major neurovascular mediators, and therefore, our current finding may suggest a novel mechanism by which pericytes regulate neurovascular homeostasis.

Disclosures: Y. Takahashi: None. **T. Maki:** None. **A.C. Liang:** None. **K. Itoh:** None. **J. Lok:** None. **N. Osumi:** None. **K. Arai:** None.

Nanosymposium

677. Blood Brain Barrier: Development and Disease

Location: 147A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:15 AM

Presentation Number: 677.02

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R01NS065089

NIH Grant P01NS055104

NIH Grant K08NS057339

Global Research Laboratory Program 2011-0021874

Japan Society for the Promotion of Science

Uehara memorial foundation

Title: Oligodendrocyte precursor cell-derived transforming-growth factor-β1 supports blood-brain barrier during development

Authors: *T. MAKI[1], J. SEO[2], M. MAEDA[3], A. C. LIANG[1], K. ITOH[1], J. LOK[1], A. TAGUCHI[3], T. MATSUYAMA[4], M. IHARA[5], K.-W. KIM[2], E. H. LO[1], K. ARAI[1]; [1]Radiology and Neurol., Massachusetts Gen. Hosp., Charlestown, MA; [2]NeuroVascular Coordination Res. Center, Col. of Pharmacy, Seoul Natl. Univ., Seoul, Korea, Republic of; [3]Dept. of Regenerative Medicine, Inst. of Biomed. Res. and Innovation, Kobe, Japan; [4]Lab. of Neurogenesis and CNS Repair, Inst. for Advanced Med. Science, Hyogo Col. of Med., Hyogo, Japan; [5]Dept. of Stroke and Cerebrovascular Diseases, Natl. Cerebral and Cardiovasc. Ctr., Osaka, Japan

Abstract: Background: The blood-brain barrier (BBB) is crucial to maintain the homeostasis of the brain. In the current view, pericytes are recruited to cerebral endothelial cells to initiate BBB formation during embryogenesis, and then astrocytes are generated over a week later to regulate BBB function during postnatal development. However, oligodendrocyte precursor cells (OPCs) are generated prior to astrocytes in the developmental stage. Therefore, in this study, we examine the roles of OPCs on the formation and regulation of BBB during development. Methods **and Results:** Firstly, we examined if OPCs exist near micro-vessels in developing brains. Immunostaining of brain sections from postnatal mice showed that micro-vessels (detected by anti-CD31 antibody) were wrapped with fine processes emanating from OPCs (detected by anti-PDGF-receptor-α antibody). As reported, astrocytes (detected by anti-GFAP antibody) also

extend elaborate processes to brain micro-vessels. To confirm that OPCs exist near cerebral endothelium, we then conducted immuno-electron microscopic observation and revealed that OPCs attach to cerebral endothelial cells via basal lamina in neonatal mouse brains. During development, transforming-growth factor-β1 (TGF-β1) plays critical roles for BBB function. Immunostaining showed that both OPCs and astrocytes expressed TGF-β1 in postnatal mice. Thus, we developed two new transgenic mouse lines with TGF-β1 deficiency specifically in OPCs or astrocytes. Compared to control mice, OPC-specific TGF-β1 knockout (*Pdgfra*^{Cre}/*Tgfb1*$^{flox/flox}$) mice showed massive cerebral hemorrhage at postnatal day 0-1, assessed by IgG staining. Concomitantly, the tight junction protein ZO-1 was degraded in the transgenic mice. By contrast, astrocye-specific TGF-β1 knockout (*GFAP*^{Cre}/*Tgfb1*$^{flox/flox}$) mice did not show apparent cerebral hemorrhage and BBB disruption at postnatal day 0-1 and day 14. Our in vitro experiments confirmed that OPC-derived TGF-β1 is supportive for the BBB integrity because conditioned media from OPCs increased expressions of tight-junction proteins and decreased endothelial permeability, which were hampered by the TGF-β-receptor inhibitor SB431542. **Conclusion:** During development, OPCs are closely located to micro-vessels in the brain and support BBB integrity by secreting TGF-β1. Therefore, besides astrocytes and pericytes, OPCs may also play an important role in supporting BBB function and homeostasis.

Disclosures: **T. Maki:** None. **J. Seo:** None. **M. Maeda:** None. **A.C. Liang:** None. **K. Itoh:** None. **J. Lok:** None. **A. Taguchi:** None. **T. Matsuyama:** None. **M. Ihara:** None. **K. Kim:** None. **E.H. Lo:** None. **K. Arai:** None.

Nanosymposium

677. Blood Brain Barrier: Development and Disease

Location: 147A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:15 AM

Presentation Number: 677.03

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH GM083165

NIH EY019492

NIH 2P30-EY005722

NIH 5P30NS061789

Title: Impairment of the blood-retina barrier and degeneration of the retinal pigment epithelium upon loss-of-functions of Ranbp2 and a subset of its Ran-GTP-binding domains

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Abstract: The retinal pigment epithelium (RPE) forms the outer blood-retinal barrier. RPE dysfunction and degeneration underpin nosologies of poorly defined etiologies and mechanisms upon genetic lesions, noxious insults or both. The pleiotropic Ran-binding protein 2 (Ranbp2) mediates diverse gene-environment interactions of distinct intrinsic and extrinsic cellular outcomes. We show that the RPE of mice, *RPE-cre::Ranbp2*$^{-/-}$, with RPE-specific *Ranbp2* ablation develop age-dependent centrifugal degeneration with pigmentary changes, syncytia, hypoplasia, nuclear atypia, and profuse choriocapillaris' leakage. RPE cells present F-actin clouds, metaloproteinase-11 but not caspase-mediated activation without apoptosis, atrophic cell extrusions into the subretinal space, peripheral RPE proliferation, and expression deregulation or subcellular delocalization of critical RPE components, such as retinoid metabolites, extracellular matrix, nuclear shuttling, cytokine and erbB signaling components. To gain mechanistic insights into RPE degeneration caused by *Ranbp2* ablation, we generated several transgenic lines of *Ranbp2* bacterial artificial chromosomes (BAC) harboring impairments of selective Ranbp2 domains in RPE or ubiquitous *Ranbp2*$^{-/-}$ background. Among these transgenic lines, only *RPE-cre::Ranbp2*$^{-/-}$*::Tg*$^{RBD2/3*-HA}$ with mutations impairing just two of the four Ran-GTP-binding domains (RBD2/3) of Ranbp2 recapitulated RPE degeneration of *RPE-cre::Ranbp2*$^{-/-}$. The pathological effects of RBD2/3 dysfunction of Ranbp2 are selective for the RPE, but not cone photoreceptors, whose function and degeneration are rescued by *Tg*$^{RBD2/3*-HA}$. RPE of *cre-RPE::Ranbp2*$^{-/-}$ and *cre-RPE::Ranbp2*$^{-/-}$*::Tg*$^{RBD2/3*-HA}$ share also proteostatic deregulation of Ran-GTPase, serotransferrin and γ-tubulin, and suppression of light-evoked electrophysiological responses. Hence, selective Ranbp2-mediated Ran-GTPase activities are vital to RPE function and survival and maintenance of blood-retinal barrier. We posit that Ranbp2-dependent Ran-GTPase dysregulation underlies pathological RPE phenoconversions upon noxious or genetic insults, and may be pathognomonic with neoplasias, where metastatic extrusions and homing promote disease progression.

Disclosures: H. Patil: None. **P.A. Ferreira:** None. **A. Saha:** None. **E. Senda:** None. **K. Cho:** None. **M. Haque:** None. **M. Yu:** None. **S. Qiu:** None. **D. Yoon:** None. **N. Peachey:** None.

Nanosymposium

677. Blood Brain Barrier: Development and Disease

Location: 147A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:15 AM

Presentation Number: 677.04

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: Osteopathic Heritage Foundation

Title: General anesthesia-induced blood-brain barrier breakdown: Potential role in delirium

Authors: *A. SARKAR[1,2,3], N. K. ACHARYA[2], M. KOSCIUK[2], J. DASH[4], C. HALE[4], E. GOLDWASER[2,3], C. JOHNSON[2,3], G. GODSEY[2,3], C. DEMARSHALL[2,3], M. FORSBERG[2], R. NAGELE[2,3,4,5];
[1]Rowan Univ., Stratford, NJ; [2]New Jersey Inst. for Successful Aging, Stratford, NJ; [3]Grad. Sch. of Biomed. Sciences, Rowan Univ., Stratford, NJ; [4]Sch. of Osteo. Medicine, Rowan Univ., Stratford, NJ; [5]Durin Technologies, Inc., New Brunswick, NJ

Abstract: The aim of this study was to investigate the possible role of general anesthesia (GA) in inducing delirium. Many elderly patients exposed to GA during surgery show post-surgical delirium, which can lead to significant long-term cognitive decline and even Alzheimer's disease. We have hypothesized that, in the elderly, some GAs may directly affect the structural and functional integrity of tight junctions occurring between adjacent brain vascular endothelial cells (BVECs), a major part of the blood brain barrier (BBB). As a result, we would predict that the integrity of the BBB in surgical patients is often disrupted during surgery by exposure to GAs. To test this possibility in animals, wild-type Sprague Dawley rats of different age groups (3-5, 10-12 and 17-19 months) were exposed to either sevoflurane or isoflurane, two commonly used GAs, and maintained in a deep sleep for 3 hours. Our goal was to create conditions analogous to that of patients having a joint replacement surgery. After 3 hours of exposure to GA, animals were either immediately euthanized by perfusion fixation or allowed to recover for 24 hours prior to fixation. Brains were recovered and prepared for scanning electron microscopy (SEM) to directly visualize the luminal surface topography of BVECs as well as their tight junction contacts that represent the BBB and immunohistochemistry (IHC) to detect leaked plasma components into the brain tissue. SEM revealed that both GAs caused dramatic changes in the luminal surface topography of BVECs. In the walls of blood vessels of GA-treated animals, most noticeably in older animals (>1.5 years), BVECs showed an increased number of well-defined circular holes at their cell margins. These holes are believed to reflect a local breakdown of tight junctions and represent a separation of adjacent cell membranes induced by the GA that provides a physical opening of the BBB that allows plasma components to gain entry into the brain tissue. This brain influx of plasma components was further supported by IHC as evidenced by the increased presence of free IgG in the brain interstitial space and IgG-positive perivascular leak clouds, primarily in older rats. By contrast, brains derived from GA-treated animals that were allowed a 24 hours recovery period prior to fixation showed restoration of a normal BVEC surface topography and a nearly complete disappearance of the gaps or holes in

the BBB. Overall, our results suggest that GAs can reversibly cause a transient BBB breakdown that is accompanied by an influx of plasma components into the brain, thereby disrupting brain homeostasis and triggering neuronal misfiring that leads to the set of symptoms that define delirium in the elderly.

Disclosures: **A. Sarkar:** None. **N.K. Acharya:** None. **M. Kosciuk:** None. **J. Dash:** None. **C. Hale:** None. **E. Goldwaser:** None. **C. Johnson:** None. **G. Godsey:** None. **C. DeMarshall:** None. **M. Forsberg:** None. **R. Nagele:** None.

Nanosymposium

677. Blood Brain Barrier: Development and Disease

Location: 147A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:15 AM

Presentation Number: 677.05

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: CIHR

CONACYT

Title: Evidence for increased microglial priming and macrophage recruitment in the dorsal anterior cingulate white matter of depressed suicides

Authors: ***S. G. TORRES PLATAS**[1], C. CRUCEANU[2], G. CHEN[1], J. DEVORAK[1], G. TURECKI[3], N. MECHAWAR[1];
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Abstract: Background: Despite all the evidence supporting the neuroinflammatory theory of depression, there is currently limited information regarding the state cerebral macrophages in individuals suffering from major depression. The aim of the present study was to examine the morphology and distribution of microglial cells and other cerebral macrophages in the dorsal anterior cingulate cortex (dACC) white matter of depressed suicides and matched nonpsychiatric controls. This region is of particular interest since we previously described the presence of hypertrophic astrocytes in depressed suicides and imaging studies have confirmed its implication in mood disorders. Methods: Using immunostained sections with the macrophage-specific marker ionized calcium binding adaptor molecule 1 (IBA1), distributions of microglial phenotypes were assessed using stereology and cell morphometry. Blood vessels were

characterized as being associated with either a high or a low density of macrophages in IBA1 and CD45-IR sections. The mRNA expression levels were quantified using real time-PCR. Results: Total densities of IBA1-immunireactive (IR) microglia were statistically similar between groups. However, the relative proportions of primed microglia were significantly increased in depressed suicides. The proportion of blood vessels surrounded by a high density of IBA1-IR macrophages was significantly higher in depressed suicides than in controls (87% vs 42%, respectively). Consistent with these findings, the mRNA expression levels of both IBA1, MCP-1, a chemokine involved in the recruitment of circulating monocytes, and Zona Occludens-1 were significantly upregulated in depressed suicides. Furthermore, the mRNA expression levels of CD45, a marker enriched in perivascular macrophages, showed a significant increased in samples from depressed suicides but the proportion of blood vessels surrounded by a high density of CD45-IR cells was similar between groups. Conclusion: Our results show evidence of microglial priming and a possible recruitment of macrophages in the dACC white matter of depressed suicides. However, we cannot exclude the possibility of other types of macrophages (including microglia) accounting for the observed increase in macrophages associated with blood vessels. Altogether, these findings suggest that the previously reported depression- and suicide-related increases in circulating pro-inflammatory cytokines may be associated with low-grade cerebral neuroinflammation involving the recruitment of circulating monocytes.

Disclosures: **S.G. Torres Platas:** None. **C. Cruceanu:** None. **G. Chen:** None. **J. Devorak:** None. **G. Turecki:** None. **N. Mechawar:** None.

Nanosymposium

677. Blood Brain Barrier: Development and Disease

Location: 147A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:15 AM

Presentation Number: 677.06

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant K08NS52550

R25NS065743

K12NS066225

Title: Endothelial tight junctions are altered in cerebral adrenoleukodystrophy

Authors: *P. L. MUSOLINO[1], S. L. JIMENEZ[2], Y. GONG[2], J. M. SNYDER[2], M. P. FROSCH[2], F. S. EICHLER[2];
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Abstract: Cerebral X-linked adrenoleukodystrophy (CALD) is a neurodegenerative disorder of childhood that results from mutations in the *ABCD1* gene and manifests as progressive inflammatory demyelination, neurological dysfunction, and death. Increased permeability to MR gadolinium at the inflammatory leading edge of the confluent lesion suggests that blood brain barrier disruption plays an important role in lesion progression. Endothelial tight junction proteins maintain restrictive permeability to inflammatory cells under normal conditions. We investigated the distribution of tight junction proteins on immunohistochemistry of human brain tissue (CALD: n=7; controls: n=2). We focused on the distribution proteins ZO-1 and CLN-5 in relationship to microglia (Iba-1) and bone marrow-derived monocytes-macrophages (CD68). Quantification of these proteins in 3 different zones (cortex, edge and core) of the lesion was standardized and assessed using Image J software. Statistical analysis was performed applying a two-way ANOVA. In CALD specimens, we found significantly increased expression of ZO-1 and CLN-5 at the inflammatory edge and in the core of the lesion compared to controls. Interestingly, Claudin-5 and ZO-1 were also found in the perivascular spaces co-localizing with CD68 and Iba-1 positive mononuclear cells within the core of the lesion. The higher expression of endothelial tight junctions proteins indicates alterations of brain endothelial cells in CALD. Redistribution of CLN-5 and ZO-1 into the extrajunctional area may be due to primary endothelial dysfunction or phagocytosis by macrophages and microglia. The extravasation of blood borne monocytes at the very edge of the lesion suggest that changes in endothelial permeability may precede lesion progression. Establishing the role of ABCD1 deficiency in the interaction between brain endothelium and inflammatory cells will increase our understanding of the pathogenic mechanisms in ALD and may identify future therapeutic targets.

Disclosures: P.L. Musolino: None. **S.L. Jimenez:** None. **Y. Gong:** None. **J.M. Snyder:** None. **M.P. Frosch:** None. **F.S. Eichler:** None.

Nanosymposium

677. Blood Brain Barrier: Development and Disease

Location: 147A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:15 AM

Presentation Number: 677.07

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: CIHR Grant 119312

Title: Comparative safety profile for the delivery of agents to the brain by magnetic resonance-guided focused ultrasound versus stereotactic needle insertion in adult rats

Authors: *M. C. TUCCI, A. BURGESS, M. A. O'REILLY, K. HYNYNEN;
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Abstract: Transient disruption of the blood-brain barrier (BBB) by magnetic resonance (MR)-guided focused ultrasound (FUS) is an emerging method for the non-invasive, highly targeted delivery of therapeutic agents such as genes and stem cells to the brain for pre-clinical research. The present study compared the safety of FUS-mediated BBB disruption with the standard stereotactic needle insertion method for agent delivery into the brain parenchyma. Adult male rats were randomly assigned to receive either FUS treatment or stereotactic needle insertion. Both treatments were administered under anaesthesia. For animals receiving FUS, T2-weighted MR images were used to target the ultrasound beam to the striatum. A non-sonicated region acted as a control for FUS treatment. A 551.5 kHz ultrasound transducer operating in burst-mode was used for ultrasound sonication, and microbubbles were injected at the onset of sonication. MR contrast agent was used to confirm BBB opening on T1-weighted images post-sonication. For animals receiving stereotactic needle insertion, a 26 gauge needle attached to a 5 μL syringe was inserted unilaterally into the striatum according to stereotactic coordinates and 1 μL of PBS was injected. The opposite hemisphere acted as a control for needle insertion. Animals were sacrificed 2 hours after receiving FUS and 48 hours after receiving stereotactic needle insertion. Overall brain morphology and red blood cell (RBC) extravasation were analysed with hematoxylin and eosin staining. RBC extravasation from animals receiving stereotactic needle insertion was extensive in the area of needle insertion in the cortex of the treated hemisphere, but there was no visible damage or RBC's away from the needle track or in the control hemisphere. Animals receiving FUS showed no evidence of tissue damage or RBC extravasation in the striatum or elsewhere in the brain. Together, these findings suggest that MR-guided FUS is a safer method than stereotactic injection for the delivery of agents to brain parenchyma. Hence, MR-guided FUS may be a more effective method for the targeted delivery of therapeutic agents to the brain for pre-clinical research.

Disclosures: **M.C. Tucci:** F. Consulting Fees (e.g., advisory boards); FUS Instruments, Inc. **A. Burgess:** None. **M.A. O'Reilly:** None. **K. Hynynen:** None.

Nanosymposium

677. Blood Brain Barrier: Development and Disease

Location: 147A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:15 AM

Presentation Number: 677.08

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: WESTON FOUNDATION GRANT

CANADIAN BLOOD SERVICES - GRADUATE FELLOWSHIP

CIHR Grant FRN 93603

Title: The delivery of immunoglobulins to the brain by focused ultrasound guided by magnetic resonance imaging: a therapeutic approach to Alzheimer's disease

Authors: *S. DUBEY[1,3], A. BURGESS[2], J. MCLAURIN[3], D. BRANCH[3], K. HYNYNEN[2,4], I. AUBERT[1,3];
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Abstract: Alzheimer's disease (AD) is characterized by several pathological hallmarks including the accumulation of amyloid beta peptides (Aβ), cell death and cognitive decline. To date, there is no treatment that halts disease progression. Intravenous immunoglobulins (IVIg), natural antibodies collected from the plasma of thousands of healthy blood donors, have been shown to improve some AD related pathologies when administered intravenously in murine models of AD. Despite these promising results, IVIg has failed to show significant cognitive improvement among AD patients in a recent Phase III clinical trial. One factor limiting the efficacy of IVIg treatments may be the restricted ability of antibodies to get through the blood brain barrier (BBB). Here, we propose using transcranial focused ultrasound (FUS), guided by magnetic resonance imaging (MRI) to temporarily increase BBB permeability and deliver IVIg to brain regions most affected by Aβ pathology. Our hypothesis is that combination of MRI-guided FUS (MRIgFUS) with IVIg therapy will improve cognitive function and decrease Aβ pathology. If effective, these data will represent a novel therapeutic approach, which uses non-invasive methods of drug delivery and may also lower the IVIg dosage required for treatment efficacy. Using a mouse model of amyloidosis, we administered IVIg intravenously, with or without application of MRIgFUS at three months of age. MRIgFUS was performed once a week, for two consecutive weeks, and was targeted to the bilateral hippocampus. After treatment, cognitive function was tested using behavioural paradigms of open field and Y-maze. Post-mortem immunohisochemistry was used to evaluate IVIg delivery to the hippocampus and changes in Aβ pathology. Our preliminary data demonstrates that IVIg is effectively delivered to the brain using MRIgFUS. Furthermore, we found that Aβ pathology is significantly reduced in mice treated with FUS + IVIg. Analysis related to cognitive functions and other hallmarks of AD are ongoing. These preliminary results suggest that FUS can improve delivery of IVIg to the brain to reduce

Aβ pathology. Optimization of IVIg drug delivery to targeted areas of the brain by FUS may be required to produce any significant changes in cognition following treatment.

Disclosures: **S. Dubey:** None. **A. Burgess:** None. **K. Hynynen:** None. **I. Aubert:** None. **J. McLaurin:** None. **D. Branch:** None.

Nanosymposium

677. Blood Brain Barrier: Development and Disease

Location: 147A

Time: Wednesday, November 19, 2014, 8:00 AM - 10:15 AM

Presentation Number: 677.09

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: CIHR grant 119312 (KH)

CIHR grant 93063 (IA)

CBCF grant (JG)

Title: Focused ultrasuond-mediated antibody delivery through the blood-brain barrier for detection and treatment of Alzheimer's disease

Authors: *A. BURGESS[1], S. DUBEY[2,3], K. SHAH[1], E. BOLEWSKA-PEDYCZAK[1], I. AUBERT[2,3], J. GARIEPY[1,4], K. HYNYNEN[1,4];
[1]Physical Sci., [2]Biol. Sci., Sunnybrook Res. Inst., Toronto, ON, Canada; [3]Lab. Med. and Pathobiology, [4]Med. Biophysics, Univ. of Toronto, Toronto, ON, Canada

Abstract: Focused ultrasound (FUS)-mediated opening of the blood-brain barrier (BBB) can promote drug delivery and reduce amyloid pathology in a mouse model of Alzheimer's disease. In this study, we assess whether amyloid antibodies labeled with MRI contrast agent and delivered by FUS can be used to identify transgenic (Tg) vs non-Tg animals using non-invasive MR imaging. Further, we determine whether the reductions in amyloid pathology by repeated FUS-mediated BBB opening with and without antibody delivery, are correlated to changes in cognitive function. 7-month-old Tg mice that exhibit behavioral deficits and amyloid pathology, and aged-matched non-Tg littermates were treated weekly with MRI-guided FUS to temporarily open the BBB in the hippocampus and cortex. Treated mice received FUS and amyloid antibodies (FUS/Ab) or FUS alone and were compared to untreated Tg and non-Tg. Mice received either Ab or Ab conjugated to a gadolinium compound (Ab-Gd). Mice were evaluated with post-treatment MRI, behavioural tests and post-mortem histology. Plaques were detected in

post-treatment MRI scans of Tg mice treated with FUS/Ab but not with FUS alone. Plaques were not observed in non-Tg mice. In the Y-Maze test, Tg mice did not perform as well as non-Tg mice. However, following FUS treatment with or without Ab, the performance of Tg mice was similar to the one of non-Tg suggesting that FUS improves cognition. No differences were observed between Tg mice treated with FUS/Ab or FUS/Ab-Gd and FUS alone. This data shows that FUS-mediated BBB opening can deliver targeted drugs to assist in non-invasive diagnosis of Alzheimer's disease. As well, repeated FUS-mediated BBB opening can improve cognition even without additional drug delivery suggesting that FUS should be considered as part of an overall therapeutic strategy for treatment of Alzheimer's patients.

Disclosures: A. Burgess: None. **S. Dubey:** None. **K. Shah:** None. **E. Bolewska-Pedyczak:** None. **I. Aubert:** None. **J. Gariepy:** None. **K. Hynynen:** None.

Nanosymposium

678. Human Reinforcement Learning: Neural Mechanisms

Location: 150B

Time: Wednesday, November 19, 2014, 8:00 AM - 10:30 AM

Presentation Number: 678.01

Topic: F.01. Human Cognition and Behavior

Title: Heterogeneity of ventromedial prefrontal cortex activity in flexible reward learning

Authors: *Z. ZHANG[1,2], A. MENDELSOHN[4], K. MANSON[2], D. SCHILLER[4,5], I. LEVY[1,2,3]; [1]Interdepartmental Neurosci. Program, Yale Univ., New Haven, CT; [2]Section of Comparative Med., [3]Dept. of Neurobio., Yale Sch. of Med., New Haven, CT; [4]Dept. of Neurosci., [5]Dept. of Psychiatry, Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Decision-making studies have repeatedly implicated the ventromedial prefrontal cortex (vmPFC) in tracking the value of rewards and punishments. At the same time, fear-learning studies have pointed to a role of the same brain area in the inhibition of previously learned responses. To disentangle these two accounts, we used an appetitive reversal-learning paradigm in human participants. In the first part of the task, the acquisition stage, participants learned that one of two colored squares (color A) was associated with monetary reward while the other (color B) was not. The acquisition stage was immediately followed by an un-signaled transition to a reversal stage, in which color B was associated with reward while color A was no longer reward-predictive. Participants' task was to estimate how likely it was for a reward to appear following each colored square presentation. fMRI data from 18 healthy participants was analyzed. Consistent with value representation, activity of a relatively dorsal region of vmPFC

was positively correlated with the magnitude of the monetary reward. Conversely, a more ventral area of vmPFC showed higher response to color A than to color B during reversal, compatible with a role of inhibiting the previously learned reward response that was no longer appropriate. Moreover, using an externally defined region-of-interest (ROI) previously associated with fear extinction we found that greater response in this area to color A compared to color B in reversal was correlated with lower reward expectancy ratings to color A compared to color B across participants. Finally, in a separate study, we applied a similar paradigm, with either food or money rewards, to normal-weight and obese participants, and found a learning impairment specific to obese women who learned with food rewards. Our findings provide direct evidence for the functional heterogeneity of the vmPFC by demonstrating simultaneous signaling of reward value and response inhibition by the dorsal and ventral regions of the vmPFC, respectively. They further suggest that a dysfunction of vmPFC may be the underlying mechanism of the observed impairment in obese women.

Disclosures: Z. Zhang: None. **A. Mendelsohn:** None. **K. Manson:** None. **D. Schiller:** None. **I. Levy:** None.

Nanosymposium

678. Human Reinforcement Learning: Neural Mechanisms

Location: 150B

Time: Wednesday, November 19, 2014, 8:00 AM - 10:30 AM

Presentation Number: 678.02

Topic: F.02. Animal Cognition and Behavior

Support: NSERC 312409-05

Title: Hierarchical control over effortful behavior in rat: A computational simulation

Authors: *C. HOLROYD[1], S. M. MCCLURE[2];
[1]Univ. of Victoria, Victoria, BC, Canada; [2]Stanford Univ., Stanford, CA

Abstract: The anterior cingulate cortex (ACC) has been implicated in a wide range of seemingly irreconcilable cognitive functions. Holroyd & Yeung (2012) proposed that many of these functions can be captured by assuming that the ACC motivates extended behaviors to achieve larger task goals. Couched within the formal theoretical framework of hierarchical reinforcement learning (HRL), this theory of ACC function holds that ACC exploits computational efficiencies afforded by collections of actions that are represented together based on their conjoint goals, called "options" in the language of HRL, that allow for behavior to be selected on the basis of

superordinate tasks that are manipulated at higher levels of abstraction (Botvinick, Niv & Barto, 2009). In contrast to alternative theories of ACC function, the HRL theory crucially accounts for behavioral sequelae of ACC damage, namely, slowed responding and reduced motor activity that, in extreme cases, is observed as akinetic mutism, or the near-absence of willed behavior despite normal motor capability. Here we instantiate the theory in a computational model of rodent behavior that makes explicit its underlying assumptions while demonstrating its internal consistency. The model proposes a hierarchical relationship between rat prelimbic cortex, ACC and the striatum, each of which control an effortful process on the basis of a common set of principles carried out at differing levels of abstraction. In the model, ACC integrates rewards across trials to learn the value of tasks, selects tasks for execution based on their learned values, and then allocates the level of control necessary for successful task performance by applying top-down control over a striatal mechanism for action selection. Because the deployment of control is assumed to deplete a conserved resource, ACC relaxes regulatory control over the striatum when events unfold smoothly and boosts control when they do not. In parallel, prelimbic cortex selects the environment for task execution and applies control over a task switching cost incurred by ACC within that environment. Computational simulations of rodent behavior in several key maze tasks implicate ACC and prelimbic cortex in regulating physical effort and cognitive switch costs, respectively.

Disclosures: C. Holroyd: None. **S.M. McClure:** None.

Nanosymposium

678. Human Reinforcement Learning: Neural Mechanisms

Location: 150B

Time: Wednesday, November 19, 2014, 8:00 AM - 10:30 AM

Presentation Number: 678.03

Topic: F.01. Human Cognition and Behavior

Title: Online feedback enhances early consolidation of motor learning and reverses recall deficits from transcranial stimulation of motor cortex

Authors: *L. WILKINSON, A. STEEL, E. MOSSHAGIAN, T. ZIMMERMANN, A. KEISLER, J. LEWIS, E. WASSERMANN;
Natl. Inst. of Neurolog. Disorders and Stroke, NIH, Bethesda, MD

Abstract: Feedback and monetary reward can enhance procedural learning, suggesting reward system involvement. Adding feedback to practice could be useful to accelerate learning/retention. Continuous theta burst transcranial magnetic stimulation (cTBS) reduces

primary motor area (M1) excitability and disrupts processing there, simulating a pathological lesion, and impairing procedural learning. To see whether feedback can overcome the effect of M1 cTBS, we delivered real or sham cTBS before two groups performed a motor sequence learning task with and without feedback. Participants were tested for sequence recall 60-minutes after cTBS administration. Sequence knowledge was indexed by changes in errors and RTs across learning/recall. Real cTBS impaired initial learning, measured as errors, and impaired sequence recall measured as RTs. Feedback improved recall indexed by RT, in both cTBS groups (Cohen's d = .76), and reversed the recall impairment observed following real cTBS (Cohen's d = .91). Only the real cTBS group in the non-feedback condition showed no evidence of explicit sequence knowledge. Feedback improves recall of implicit and explicit procedural knowledge and can protect knowledge against the effect of M1 inhibition. Adding feedback and monetary reward/punishment to procedural learning may help overcome retention impairments or accelerate training in clinical and other settings.

Disclosures: L. Wilkinson: None. **A. Steel:** None. **E. Mosshagian:** None. **T. Zimmermann:** None. **A. Keisler:** None. **J. Lewis:** None. **E. Wassermann:** None.

Nanosymposium

678. Human Reinforcement Learning: Neural Mechanisms

Location: 150B

Time: Wednesday, November 19, 2014, 8:00 AM - 10:30 AM

Presentation Number: 678.04

Topic: F.01. Human Cognition and Behavior

Title: Adaptive effort allocation via the limbic loop

Authors: *T. VERGUTS[1], E. VASSENA[1], M. SILVETTI[2];
[1]Psychology, [2]Ghent Univ., Ghent, Belgium

Abstract: Despite its importance in everyday life, the computational nature of effort exertion remains poorly understood. We propose an abstract model on effort exertion obtained from optimality considerations, and a neurobiological approximation to the optimal model. Both are couched in the framework of reinforcement learning. It is shown that choosing when or when not to exert effort, depending on its benefits and costs, can be adaptively learned. In the neurobiological model, the limbic loop comprising anterior cingulate cortex and ventral striatum in the basal ganglia provides a "boosting input" to cortical stimulus-action pathways whenever this is valuable. We demonstrate that the neurobiological model approximates optimality. Next, we simulate physical effort tasks. In line with empirical work, impairing the model's

dopaminergic pathway leads to apathetic behavior; moreover, the model balances costs and benefits in variable-interval schedules, leading to hyperbolic responding. Finally, we apply the model to a congruency (e.g., Stroop) task paradigm from the cognitive control literature. We demonstrate that the model exhibits congruency and sequential congruency effects (i.e., adapts after difficult trials). Thus, we conceptually unify the exertion of physical and cognitive effort, studied across a variety of literatures (e.g., motivation and cognitive control) and animal species.

Disclosures: **T. Verguts:** None. **E. Vassena:** None. **M. Silvetti:** None.

Nanosymposium

678. Human Reinforcement Learning: Neural Mechanisms

Location: 150B

Time: Wednesday, November 19, 2014, 8:00 AM - 10:30 AM

Presentation Number: 678.05

Topic: F.01. Human Cognition and Behavior

Support: BBSRC BB/I019847/1

Title: Feedback processing is adaptive to information on volatility

Authors: ***A.-M. SCHIFFER**[1], F. WASZAK[2], N. YEUNG[1];
[1]Exptl. Psychology, Univ. of Oxford, Oxford, United Kingdom; [2]Univ. Paris Descartes, Paris, France

Abstract: Successful interaction with volatile environments requires adaptive responding to changing contingencies. This faculty is particularly important when contingencies in the environment are non-deterministic: Unexpected events sometimes indicate true changes in the world (requiring a change in response strategy) but sometimes reflect chance occurrence (which must be discounted to maintain stable behavior). While recent studies have addressed how humans integrate feedback to adapt to volatile environments, very few have assessed how explicit information and instruction may be integrated with experience to guide behavior. We therefore conducted a series of EEG studies to investigate how high-level information affects behavior and neurophysiological correlates of learning. Participants performed a reversal-learning task in which both feedback and instruction on current volatility were probabilistic. We found that participants integrated these two sources of information and reversed stimulus-response mappings after fewer trials with surprising feedback if they had been told that volatility was high. Participants' behavior was predicted by a Bayesian model that integrated trial-by-trial feedback to decide between probable states of the environment. EEG analysis showed that the

feedback-related negativity (FRN) was significantly modulated by the information theoretic measure of surprise, derived from the Bayesian model. FRN amplitude was higher if participants were told that volatility was high, compared to when they were told that volatility was low, even when conditions were matched in terms of actual experienced outcomes. Meanwhile, the anticipatory stimulus-preceding negativity (SPN) indicated greater attention to feedback when participants were instructed towards high compared to low volatility. As unexpectedness and informative value are often conflated, these novel findings have important implications for our understanding of hierarchies of information in learning. Modulation of the SPN indicates a preparatory process directed at informative feedback depending on its relevance to adaptive behavior. The increased FRN under these conditions suggests a specific coding of unexpectedness when it indicates relevant changes. Hierarchical information on volatility is taken into account to adaptively deal with uncertainty elicited by unexpected events, and this process is reflected in ERP correlates of stimulus anticipation and processing.

Disclosures: **A. Schiffer:** None. **F. Waszak:** None. **N. Yeung:** None.

Nanosymposium

678. Human Reinforcement Learning: Neural Mechanisms

Location: 150B

Time: Wednesday, November 19, 2014, 8:00 AM - 10:30 AM

Presentation Number: 678.06

Topic: F.01. Human Cognition and Behavior

Support: NSF BCS 1150708

Title: Avoidance goals influence striatal processing of negative feedback during learning

Authors: ***S. D. SWANSON**, E. TRICOMI;
Rutgers Univ., Newark, NJ

Abstract: Feedback about performance accuracy is frequently used by educators, clinicians, parents, and others to help promote learning, cultivate new skills, and reduce maladaptive behaviors. Motivation is a crucial but often neglected factor in determining how effectively feedback can facilitate learning. This fMRI study was designed to explore the effects of avoidance motivation on striatal feedback responses during learning. Avoidance goals motivate individuals to avoid undesired outcomes such as poor performance. We hypothesized that individuals experiencing high levels of avoidance goals relative to other types of goals would find negative feedback more threatening and exhibit exaggerated neural punishment responses to

negative feedback. Participants received a below-average percentile ranking after taking a bogus (but believable) intelligence test to induce a state of threat (Laws & Rivera, 2012) , which amplifies the effects of avoidance goals on performance. Task-specific avoidance goals were assessed via self-report at the end of the study, and feedback-based learning was probed using a paired associate word learning task, which is known to engage the striatum (e.g., Tricomi & Fiez, 2008). During learning, individual differences in the bias toward avoidance goals showed a negative correlation with activation at the time of negative feedback in both the ventral striatum ($p=.008$) and caudate nucleus ($p=.004$). In other words, individuals who were biased toward avoidance goals exhibited larger decreases in striatal signal following negative feedback, which is suggestive of an exaggerated punishment response. Since avoidance goals have been linked with adverse outcomes in a variety of achievement domains, these findings suggest that exaggerated punishment signals in the striatum may reflect a maladaptive response to feedback during learning.

Disclosures: S.D. Swanson: None. **E. Tricomi:** None.

Nanosymposium

678. Human Reinforcement Learning: Neural Mechanisms

Location: 150B

Time: Wednesday, November 19, 2014, 8:00 AM - 10:30 AM

Presentation Number: 678.07

Topic: F.01. Human Cognition and Behavior

Support: James S McDonnell Foundation (710373)

Title: Using olfactory aversive conditioning during human sleep to treat addiction

Authors: *A. ARZI, Y. HOLTZMAN, P. SAMNON, N. ESHEL, N. SOBEL;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Cigarette smoking is an addictive behavior associated with significant morbidity and mortality. Efforts to treat addiction using aversive conditioning have seen only limited success. The effectiveness of conditioning may be greater when it is implicit rather than explicit. One implicit path is conditioning during sleep. Therefore, we set out to test the hypothesis that olfactory aversive conditioning during sleep can reduce smoking. A daily smoking diary detailing the number of smoked cigarettes was completed by 76 smokers wanting to quit (mean age = 28.7 ±5.2 years, 26 F) during 7 days before and 7 days after a 1 night or day experiment. The experiment consisted of olfactory aversive partial trace conditioning between cigarette odor

and profoundly unpleasant odors. The conditioned (cigarette odor) and non-conditioned (unpleasant odors) stimuli were partially reinforced at a ratio of 2:1; on reinforced trials, a 5-s cigarette odor was paired with a 3-s unpleasant odor (either Ammonium Sulfide (AmSu) or Rotten Fish (RF)). On nonreinforced trials, cigarette odor was generated without an ensuing unpleasant odorant (cigarette odor alone). Stimuli were generated in blocks of 30 trials (10 trials reinforced with AmSu, 10 reinforced with RF, and 10 nonreinforced with cigarette odor only, randomized across the block). An initial experiment limited to either Stage 2 sleep or wake revealed that olfactory aversive conditioning during Stage 2 sleep significantly reduced smoking by 47.9% ± 38.6%, and that the reduction lasted several days (all $t(10) > 2.3$, $p <$ FDR α). Conditioning during wake did not reduced smoking (all $t(9) < 1.45$, $p >$ FDR α). To test whether the reduction in smoking stemmed from the conditioning between cigarette odor and unpleasant odors, or merely from the presentation of either unpleasant odors or cigarette odor we conducted two control experiments: 1) the same experimental procedure without cigarettes odor 2) Cigarette and aversive odors randomly interspersed. There was no significant change in smoking following these control experiments (all $t(10) < 2.6$,$p >$ FDR α). To conclude, a single night of olfactory aversive conditioning between cigarette odor and unpleasant odors during sleep can reduce smoking.

Disclosures: **A. Arzi:** None. **Y. Holtzman:** None. **P. Samnon:** None. **N. Eshel:** None. **N. Sobel:** None.

Nanosymposium

678. Human Reinforcement Learning: Neural Mechanisms

Location: 150B

Time: Wednesday, November 19, 2014, 8:00 AM - 10:30 AM

Presentation Number: 678.08

Topic: F.01. Human Cognition and Behavior

Support: ARC FL0992409

Title: Reduced causal awareness associated with right pallidal shape changes in youth during the early phases of psychiatric disorders

Authors: *K. R. GRIFFITHS, J. LAGOPOULOS, D. F. HERMENS, I. B. HICKIE, B. W. BALLEINE;
Brain and Mind Res. Inst., Univ. of Sydney, Sydney, Australia

Abstract: Cognitive impairments contribute significantly to disease burden in young patients presenting in the early phases of major psychiatric disorders. In particular, impairments in causal awareness may lead to suboptimal decision-making, resulting in poorer life outcomes. Here we investigated the causal sensitivity of 94 help-seeking youth with an admixture of psychiatric illnesses, and 20 demographically similar healthy controls. Using voxel-based morphometry (VBM), volumetrics, shape analysis, probabilistic tractography and tract-based spatial statistics (TBSS), we investigated the relationship between grey and white matter structural integrity and causal awareness. Whilst patients in the upper tertile of causal awareness were comparable with controls, those in the lower tertile had reduced right pallidal size. More specifically, shape analysis revealed that vertices on the dorsolateral pallidal surfaces were correlated with levels of causal sensitivity. Probabilistic tractography revealed afferent and efferent connections to the affected parts of the pallidum. White matter microstructural measurements along these pathways however did not vary linearly with causal awareness. We conclude that causal awareness deficits are associated with localized changes within the right pallidum. This occurs across psychiatric diagnoses, and may predictive of those at greater risk for poorer functional outcomes.

Disclosures: K.R. Griffiths: None. **J. Lagopoulos:** None. **D.F. Hermens:** None. **I.B. Hickie:** None. **B.W. Balleine:** None.

Nanosymposium

678. Human Reinforcement Learning: Neural Mechanisms

Location: 150B

Time: Wednesday, November 19, 2014, 8:00 AM - 10:30 AM

Presentation Number: 678.09

Topic: F.01. Human Cognition and Behavior

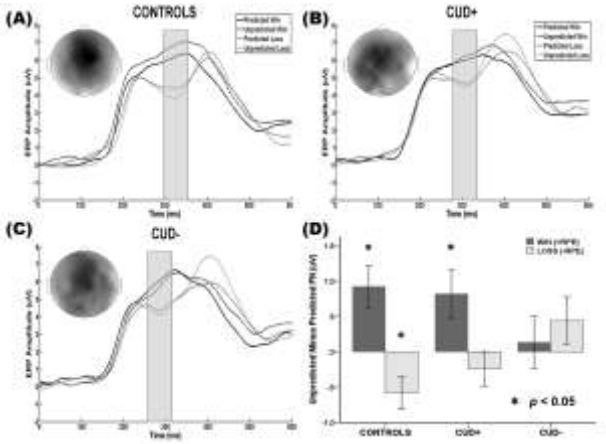
Support: NIDA F32DA033088-01

NIDA R01DA023579

Title: Tracking bidirectional prediction error signals and their disruption in cocaine addiction using feedback negativity

Authors: *M. A. PARVAZ[1], A. B. KONOVA[2], G. H. PROUDFIT[2], S. J. MOELLER[1], P. MALAKER[1], N. ALIA-KLEIN[1], R. Z. GOLDSTEIN[1];
[1]Psychiatry, Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]Psychology, Stony Brook Univ., Stony Brook, NY

Abstract: Learning can be guided by unexpected success or failure, signaled via dopaminergic positive (+RPE) and negative reward prediction error (-RPE) signals, respectively. Although empirical evidence is limited, RPE signaling is thought to be impaired in drug addiction. To fill this gap, we studied the feedback negativity (FN), an event-related potential component that is sensitive to both reward and the violation of expectation, as a measure of RPE signaling. We examined the FN in 25 healthy controls, in 25 individuals with cocaine use disorder (CUD) who tested positive for cocaine (CUD+), indicating cocaine use within the past 72 h, and in 25 CUD who tested negative for cocaine (CUD-) indicating less recent use. Participants performed a gambling task where they predicted if they would win/lose money on a trial-by-trial basis given three known probabilities of winning (25%, 50%, or 75%). A significant interaction between prediction, outcome and group [$F(2,72)=5.58$, $p=0.006$] revealed intact +RPE and -RPE signaling in controls [Unpredicted Win > Predicted Win; $t(24)=3.12$, $p=0.005$; Unpredicted Loss < Predicted Loss; $t(24)=2.50$, $p=0.020$, respectively]. Both CUD subgroups, however, did not show modulation of the FN on loss trials (i.e., -RPE; $p>0.203$), suggesting an impaired -RPE signaling in these individuals. Moreover, unlike CUD- ($p=0.719$), CUD+ showed increased FN amplitude for unexpected wins compared to expected wins [$t(24)=2.38$, $p=0.026$], as indicative of intact +RPE signaling, similar to controls. Lastly, the +RPE mediated FN modulation was negatively correlated with days of current abstinence ($r=-0.402$; $p=0.008$) across all CUD showing that longer abstinence exacerbates +RPE signaling in CUD. Thus, using the FN, the current study for the first time directly documents differential RPE deficits in CUD as a function of recent drug use pattern. Abnormal ±RPE computation specifically in CUD- suggests a unique vulnerability that may impair adaptive, reinforcement-based goal-driven behavior especially in non-recent users, which could threaten attempts at sustained abstinence.



Disclosures: **M.A. Parvaz:** None. **A.B. Konova:** None. **G.H. Proudfit:** None. **S.J. Moeller:** None. **P. Malaker:** None. **N. Alia-Klein:** None. **R.Z. Goldstein:** None.

Nanosymposium

678. Human Reinforcement Learning: Neural Mechanisms

Location: 150B

Time: Wednesday, November 19, 2014, 8:00 AM - 10:30 AM

Presentation Number: 678.10

Topic: F.01. Human Cognition and Behavior

Title: Mid-frontal origin of dopamine temporal difference dynamics

Authors: *M. SILVETTI, T. VERGUTS;
Ghent Univ., Ghent, Belgium

Abstract: The majority of dopaminergic neurons in the ventral tegmental area (VTA) respond initially to unconditioned rewarding stimuli, but after conditional training, to arbitrary stimuli that are predictive of incoming reward (Schultz et al., 1986). This dynamics is typically interpreted in terms of a specific class of reinforcement learning algorithms, called temporal difference (TD) learning (Schultz et al., 1997). This approach suggested that VTA computes TD signals to formulate reward expectations. Nonetheless several empirical findings challenged the classical TD-related explanation of VTA dynamics (Pan et al., 2005). Moreover, it remained unclear whether such a temporal dynamics was computed directly by the VTA or originated from other brain structures. Here we propose a novel neuro-computational account of VTA dopaminergic dynamics. Our findings are based on an already existent neural model concerning reinforcement learning operations in the anterior cingulate cortex, the VTA and their interactions: the Reward Value Prediction Model (RVPM, Silvetti et al., 2011). We propose that VTA dynamics derives from processing of signals afferent from the ACC, with the purpose of training yet other brain areas. To test this hypothesis, we ran four computer simulations where we administered to the RVPM several sessions of classical conditioning. Through the ACC-VTA interaction, we successfully managed to reproduce the most relevant findings on dopamine dynamics, like dopamine shifting from reward period to cue period, summation and overshadowing effects, blocking, and dopamine dynamics in paradigms with multiple cues. These results allowed us to provide a comprehensive set of simulations about dopaminergic activity of mammalian brainstem.

Disclosures: **M. Silvetti:** None. **T. Verguts:** None.

Nanosymposium

679. Human Cognition: White Matter and Functional Connectivity

Location: 146C

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 679.01

Topic: F.01. Human Cognition and Behavior

Support: NIH grant 5R37AG-006265-27

NIH grant 4 R00AG-036818-04

Title: Age-related decreases in regional white matter integrity predict reduced neural modulation to task processing demands: A combined DTI and fMRI lifespan aging study

Authors: *K. M. KENNEDY, J. R. RIECK, P. EVANS, D. C. PARK;
Behavioral & Brain Sci., Univ. Texas, Dallas, DALLAS, TX

Abstract: Aging is associated with changes in brain structure and function, but the association between these changes is poorly understood. One possibility is that maintained integrity of the brain's white matter structural connections as we age helps support brain function. In a large lifespan sample of healthy adults aged 20-89 (N=308) we measured regional white matter integrity via Diffusion Tensor Imaging (DTI) tractography and measured neural activation to a semantic judgment with easy and hard conditions via fMRI. fMRI results revealed 9 clusters of age-related decreases in modulation to increased task demands (hard vs easy) largely in cognitive control regions (e.g., bilateral dlPFC, vlPFC, PPC, caudate/thalamus, cerebellum). We investigated the underlying role of age-related regional white matter connectivity degradation in 5 white matter tracts [(genu and splenium of the corpus callosum, and bilateral uncinate (UF), superior longitudinal (SLF) and inferior longitudinal fasciculi (ILF)] in their relation to age-related modulation declines in these 9 functional ROIs. We found that age-related reduction in white matter tract integrity was significantly associated with reduced modulation of activation to task demand in the right hemisphere ROIs selectively. Specifically, decreased modulation to task demand in posterior parietal cortex ROI (angular/superior/inferior parietal cortex) was associated with poorer white matter integrity in the splenium of the corpus callosum; decreased modulation in inferior/middle frontal ROI was associated with degraded white matter in the left ILF; decreased orbito-frontal/insula/inferior frontal modulation was associated with degraded white matter in the splenium, left ILF, and left uncinate fasciculus; decreased modulation in cerebellum (Crus 2) was associated with degradation to genu of the corpus callosum and left uncinate; finally decreased modulation in the caudate/thalamus was associated with age-related degradation in the splenium, left ILF, and bilateral uncinate tracts. We conclude that decreased modulation of activation with age to increased task demands is supported by the integrity of the white matter connections underlying these regions of the cognitive control system in specific, highly relevant anatomical circuitry.

Disclosures: **K.M. Kennedy:** None. **J.R. Rieck:** None. **P. Evans:** None. **D.C. Park:** None.

Nanosymposium

679. Human Cognition: White Matter and Functional Connectivity

Location: 146C

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 679.02

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R37 AG024102

NIH Grant R03AG045494

NIH Grant P01AG043362

NIH Grant F32AG042228

NIH Grant R00 AG036818

NIH Grant R00 AG036848

Title: Longitudinal Change in association white matter tract integrity is related to cognition in midlife and older adults: Findings from Seattle Longitudinal Study

Authors: *S. L. WILLIS[1], K. M. KENNEDY[3], A. L. GROSS[4], P. R. A. ROBINSON[5], P. RAST[6], D. MCLAREN[7], K. M. RODRIGUE[3], L. BARRERA-MARTINEZ[5], K. SCHAIE[2]; [2]Psychiatry and Behavioral Sci., [1]Seattle Longitudinal Study. Univ. of Washington, SEATTLE, WA; [3]Brain and Behavioral Sci., Univ. of Texas at Dallas, Dallas, TX; [4]Epidemiology, Johns Hopkins Univ., Baltimore, MD; [5]Integrated Brain Imaging Ctr., Univ. of Washington, SEATTLE, WA; [6]Dept. of Psychology, Univ. of Victoria, Victoria, BC; [7]Dept. of Neurol., Harvard Med. Sch., Boston, MA

Abstract: Longitudinal studies of white matter (WM) connectivity are sparse and none have examined associations with cognitive decline. It is unknown how within-person changes in white matter degradation relate to aging of cognitive performance. We examined 4-year change in white matter integrity at two-year intervals (2008, 2010, 2012) in an adult sample of N = 184 Seattle Longitudinal Study participants (Mage = 67, range 50-82 at first scan). We further examined the association between WM integrity and 28-yr cognitive change in 6 abilities (1984 - 2012) via parallel processing multivariate multilevel models. Tract data were processed with

FSL software (eddy correction, skull stripping, tensor computation). Probtrackx was used for probabilistic fiber tracking in native diffusion space using starting and wayfinding seeds for each tract of interest. FA, MD, AD and RD were measured in 5 association tracts: superior longitudinal fasciculus (SLF), inferior longitudinal fasciculus (ILF), uncinate fasciculus (UNC), fornix (FX) and cingulum (CG). Multivariate multilevel models using a stepwise modeling procedure examined longitudinal change. A baseline model of change in association tracts was followed by introduction of explanatory variables (APOE genotype, hypertension) to predict individual differences in rate of change in WM integrity. Reliable age differences ($p < .05$) in FA and RD occurred in all five association tracts, with AD age differences in SLF, UNC, FNX. All tracts showed statistically significant change over 4 years with radial regions (e.g. myelin) more affected than axial: RD(ILF,SLF,UNC); FA (ILF, UNC, CG), AD (ILF,SLF) with moderation by APOE e4 (SLF,UNC,FNX); HBP (ILF). White matter connectivity in association tracts was broadly associated with declines in cognitive performance over 28 years in all domains examined. Executive function and number ability 28 years ago was associated with ILF FA level. We also found change in ILF FA over 4 years was associated with 28 year change in spatial orientation. Our findings show measurable degradation of white matter connectivity of the association tracts of the brain that is associated with cognitive decline, lending support to "cortical disconnection" theories of cognitive aging

Disclosures: S.L. Willis: None. **K.M. Kennedy:** None. **A.L. Gross:** None. **P.R.A. Robinson:** None. **P. Rast:** None. **D. McLaren:** None. **K.M. Rodrigue:** None. **L. Barrera-Martinez:** None. **K. Schaie:** None.

Nanosymposium

679. Human Cognition: White Matter and Functional Connectivity

Location: 146C

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 679.03

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant P01 AG036694

NIH Grant R01 AG034556

NIH Grant P41 EB015896

NIH Grant K01 AG040197

Title: Functional connectivity in multiple cortical networks is associated with cognitive performance in older adults

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Abstract: Resting state functional connectivity MRI has become a widely-used tool for measuring integrity in large-scale cortical networks. This study examined multiple cortical networks using Template-Based Rotation (TBR), a method that applies a priori network and nuisance component templates defined from an independent dataset to test datasets of interest. A priori templates were applied to a test dataset of 66 younger (ages 18-32) and 276 older adults (ages 65-90) from the Harvard Aging Brain Study to examine the relationship between multiple large-scale cortical networks and cognition. Factor scores derived from a neuropsychological battery represented processing speed, executive function, and episodic memory. Resting-state BOLD data were acquired in two six-minute acquisitions on a 3-Telsa scanner, screened for data quality including motion, and processed with TBR to extract individual-level metrics of network integrity in multiple cortical networks. Age differences between younger and older adults were observed in the integrity of multiple cortical networks and in cognition. Within the older adults, integrity in multiple large-scale cortical networks was positively related to all cognitive domains, with a composite measure of general connectivity positively associated with general cognitive performance. Controlling for the correlations between networks, only a positive relation between the fronto-parietal control network and executive function was significant, suggesting specificity in this relationship. These results extend prior work demonstrating that functional connectivity metrics in multiple cortical networks are associated with individual variation in cognition, and further suggest that TBR may be a useful tool for measuring relationships between reduced network integrity and cognition during aging.

Disclosures: T. Hedden: None. **E.E. Shaw:** None. **A.P. Schultz:** None. **R.L. Buckner:** None. **R.A. Sperling:** None.

Nanosymposium

679. Human Cognition: White Matter and Functional Connectivity

Location: 146C

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 679.04

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R37AG024102

NIH Grant F32AG042228

NIH Grant P01AG043362

NIH Grant R03AG045494

NIH Grant R00AG038484

NIH Grant R00AG036818

Title: Longitudinal thinning in association cortices is related to cognition in healthy middle aged and older adults: findings from the seattle longitudinal study

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Abstract: The majority of brain aging research utilizes cross-sectional data to examine age-related differences in brain structure and cognition. Longitudinal studies that not only investigate neural change within individuals, but also the association between neural and cognitive change are sparse. We examined 4-year longitudinal change in cortical thickness, at two-year intervals (2006 - 2010), in a healthy adult sample of 163 Seattle Longitudinal Study participants (Mean baseline age = 67, range 52-87) on a Philips 3T Achieva scanner, using multivariate multilevel modeling. We further examined the association between cortical thinning and cognitive change (4 visits between 1984 - 2005) using parallel longitudinal growth models. Cortical thickness estimation was performed with FreeSurfer v5.1. Cortical thickness values for each of the 68 parcels were extracted for each participant and timepoint. Multivariate multilevel models using a stepwise modeling procedure examined longitudinal change of association cortices. A baseline model of change in cortical thickness in frontal, parietal, temporal and occipital regions was followed by including explanatory variables (APOE haplotype, hypertension) to explain individual differences in rate of cortical thinning. Statistically significant age-related decreases in

cortical thickness were found in all regions except occipital cortex. We also found significant atrophy over time in parietal and occipital lobes; the occipital lobe evidenced accelerated thinning with higher age. In frontal and temporal regions, atrophy was accelerated in APOE e4 carriers. Hypertension was associated with reduced cortical thickness in the parietal and temporal lobes. Reliable individual differences in rate of atrophy were observed for all regions. Additionally, the association of rate of change across 28-years in 6 cognitive domains was compared to the rate of atrophy over 4 years. Longitudinal parallel process latent growth models were estimated for both cognitive domains and cortical thickness (frontal, parietal,temporal, cingulum, and occipital) to pair each cognitive domain and cortical region, allowing correlations among latent intercepts and slopes. Atrophy in frontal and temporal regions was associated with both baseline levels and change in perceptual speed; while atrophy in the frontal, temporal and cingulum regions was associated with working memory. We conclude that brain-cognition relationships are complex and extend across middle and late life.

Disclosures: D.G. McLaren: None. **P. Rast:** None. **A.L. Gross:** None. **K.M. Kennedy:** None. **K.M. Rodrigue:** None. **P.R.A. Robinson:** None. **L. Barrera-Martinez:** None. **K.W. Schaie:** None. **S.L. Willis:** None.

Nanosymposium

679. Human Cognition: White Matter and Functional Connectivity

Location: 146C

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 679.05

Topic: F.01. Human Cognition and Behavior

Support: National Institutes of Health grant: MH060941

Title: Age-related dedifferentiation of intrinsic functional connectivity both within and between the default and dorsal attention networks

Authors: *W. D. STEVENS[1], R. N. SPRENG[2], J. D. VIVIANO[1], D. L. SCHACTER[3]; [1]Psychology, York Univ., Toronto, ON, Canada; [2]Human Develop., Cornell Univ., Ithaca, NY; [3]Psychology, Harvard Univ., Cambridge, MA

Abstract: Recent work indicates that the default and dorsal attention networks of the human brain are critically involved in internally and externally directed cognition, respectively. Anticorrelation between these two networks is a central feature of human functional brain organization, serving as a critical neural substrate for flexibly allocating attentional resources

necessary for healthy cognitive function. Age-related cognitive decline is associated with impaired modulation of default network activity. However, it remains unclear if the robust pattern of anticorrelation is preserved in older adulthood. We recently demonstrated that a breakdown of this opposing connectivity is apparent during task-related functional coupling. Here, we hypothesized that impaired task-related modulation of brain activity reflects underlying dedifferentiation in the intrinsic functional network architecture of these two systems, which would manifest as decreased intra-network functional connectivity and/or decreased anticorrelation between the default and dorsal attention networks. To test this, resting-state functional connectivity (RSFC) analyses were conducted on fMRI data from a large group of healthy young (n = 57) and older (n = 75) adults. Several fMRI data processing techniques aimed at reducing or eliminating potential artifacts associated with differences in head motion across the young and older groups were employed and compared. A whole-brain RSFC analysis indicated that although both the young and older adults showed a negative correlation between the default and dorsal attention networks, this relationship was somewhat attenuated in the older adults. ROI-based RSFC analyses revealed a double dissociation, such that in the older adults, relative to the younger adults, while there was weaker within-network coupling among nodes of both the default and dorsal attention networks, there was increased cross-network coupling between particular nodes of these two networks. Taken together, these results revealed a robust pattern of dedifferentiation in older adults, relative to young adults, characterized by decreased intrinsic functional connectivity within the default and dorsal attention networks, and decreased anticorrelation between these networks. The results are consistent with the idea that age-related cognitive decline is associated with a breakdown in the intrinsic functional architecture within and among large-scale brain networks.

Disclosures: W.D. Stevens: None. **R.N. Spreng:** None. **J.D. Viviano:** None. **D.L. Schacter:** None.

Nanosymposium

679. Human Cognition: White Matter and Functional Connectivity

Location: 146C

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 679.06

Topic: F.01. Human Cognition and Behavior

Support: NIA grant 1R01AG039103

Title: Age-related changes in the relationship between recollection accuracy and recollection-related increases in functional connectivity

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Abstract: Recollection involves retrieving specific contextual details about a prior event. Functional neuroimaging studies have identified several brain regions that are consistently active during successful recollection, referred to as the 'core recollection network.' Normal aging is associated with decline in recollection accuracy; however, the changes in brain function that underlie this decline are unclear. We have previously found that, in young adults, members of the core recollection network show enhanced functional connectivity both with other core regions, and regions outside of the network, during successful relative to unsuccessful recollection. Importantly, the magnitude of these connectivity increases correlated across individuals with recollection accuracy. Here, we used functional magnetic resonance imaging (fMRI) to investigate the effects of age on recollection-related connectivity changes. Young (18-30 years, N=36), middle-aged (43-55 years, N=36), and older adults (63-76 years, N=64) were scanned while making associative recognition judgments (discriminating 'intact' from 'rearranged' test pairs) about previously encoded word pairs. As is typical in such studies, associative recognition accuracy declined monotonically across the age groups. We identified brain regions that were more active during successful relative to unsuccessful recollection using standard univariate analyses. We then applied psychophysiological interactions (PPI) analyses to identify regions that showed recollection-related connectivity increases with five different recollection-sensitive seeds, each a member of the core network. Consistent with our previous findings, a widely distributed, overlapping set of brain regions showed increased recollection-related connectivity with all five seeds. These regions were highly consistent across age groups, with no significant age-related differences in the magnitude of the connectivity increases. There was, however, a significant age-related difference in the extent to which the change in connectivity was related to recollection accuracy. This relationship was significantly stronger in the young compared to the middle-aged and older adults, and for some seeds, the relationship was significantly greater for middle-aged compared to older adults. These results suggest that although older and younger adults show similar patterns of increased connectivity associated with successful recollection, the change in connectivity might reflect a mechanism that benefits recollection performance to a greater extent in younger than in older individuals.

Disclosures: D.R. King: None. **M. de Chastelaine:** None. **M.D. Rugg:** None.

Nanosymposium

679. Human Cognition: White Matter and Functional Connectivity

Location: 146C

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 679.07

Topic: F.01. Human Cognition and Behavior

Support: Friends of BrainHealth 2011 Distinguished New Scientist Award

NIA Grant AG029523

NIA Grant AG038299

Title: Microstructural estimates of grey matter and white matter age differentially predict lifespan variability in fluid and crystallized intelligence

Authors: *E. SHOKRI-KOJORI[1], I. J. BENNETT[2], D. C. KRAWCZYK[1], B. RYPMA[1]; [1]The Univ. of Texas at Dallas, Ctr. for BrainHealth, Dallas, TX; [2]Univ. of California, Irvine, Irvine, CA

Abstract: Fluid intelligence (G*f*; ability to reason in novel conditions) declines over the lifespan whereas crystallized intelligence (G*c*; ability to access past knowledge and experience) improves, yet the underlying neurobiological correlates remain poorly understood. We hypothesized that different tissue types, in particular grey matter (GM) and white matter (WM), age differently and account for different aspects of age-related variability in G*f* and G*c*. Diffusion Tensor Imaging (DTI) as well as high resolution anatomical data were acquired on 100 individuals ranging in age from 18 to 78 years old. Microstructural properties within each voxel were estimated using DTI indices: fractional anisotropy (FA), axial diffusivity (AD), and radial diffusivity (RD). FA, AD, and RD voxel distributions were extracted within anatomically defined masks of GM and WM. Per participant, tissue-specific distribution moments (i.e., mean, variance, and skewness) were then estimated for brain-wide characteristics of FA, AD, and RD. For each tissue type, stepwise regression was performed to identify relevant predictors of chronological age using FA, AD, and RD distribution moments and volume information. Next, by using a leave-one-out approach, tissue-specific predictors of chronological age were used to estimate GM and WM age. GM age accounted for 74% of chronological age variance, while WM age accounted for 58% of chronological age variance. The rate of GM and WM aging was further assessed before and after age 40 (the median sample age; MSA). Stepwise regression showed that when GM and WM regressors were simultaneously used to predict chronological age, only GM regressors significantly accounted for age variance pre-MSA. In contrast, only WM regressors were significant predictors of age variance post-MSA. These results suggest that GM ages more rapidly than WM pre-MSA, but WM ages more rapidly than GM post-MSA. Furthermore, measures of G*f* and G*c* were obtained on a subsample of 20 younger and 20 older participants. As expected, G*f* scores were higher in the younger participants, while the older

participants showed higher G*c* scores ($p < 0.05$). Estimates of GM and WM age were used to predict G*f* and G*c* abilities. Variability in G*f* pre-MSA was only correlated with GM age, while the variability in G*c* post-MSA was only correlated with WM age ($p < 0.05$). These results support our prediction that GM and WM age at different rates and that each is differentially sensitive to age-related variability in fluid and crystallized abilities, respectively. These findings provide new insights into neurobiological mechanisms of changes in intellectual abilities over the lifespan.

Disclosures: E. Shokri-Kojori: None. **I.J. Bennett:** None. **D.C. Krawczyk:** None. **B. Rypma:** None.

Nanosymposium

679. Human Cognition: White Matter and Functional Connectivity

Location: 146C

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 679.08

Topic: F.01. Human Cognition and Behavior

Title: Hierarchical neural basis of memory in the aging human brain

Authors: S. PUNZELL[1,2], ***E. DOWLATI**[1,2], S. ADAMS[2], R. MORAN[2];
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Abstract: Declarative memory, which involves higher order cortical regions, is most vulnerable to aging decline. Older adults have been shown to have both increased prefrontal cortex activity in previous memory studies. Analysis of the causal relationship between neural activity in these areas during memory encoding in aged and young brains has yet to be determined. Our aim was to determine whether or not older brains elicit deeper contextual generalizations, via top-down activity over detail related, bottom-up pathways during memory tasks. We utilized an image pair recognition task in both a young and old cohort. Blood-oxygen level dependent (BOLD) responses were measured from fMRI during memory encoding as participants were asked two different questions (a low and high context prime) constructed to elicit brain activity in top-down or bottom-up related areas. A two-way analysis of variance (ANOVA) was utilized to analyze our behavioral results. In recall percent correct, young subjects scored higher than old ($P = 0.0003$), and detail-associated pairs had higher percent than context-associated ($P = 0.02$). fMRI scans were analyzed and global maxima in the right calcarine sulcus (rCS) and right inferior frontal gyrus (rIFG) were located. Specifically rCS responded with greater activity to detail-

associated compared to context-associated items, while the rIFG displayed different activity dependent on the encoding block type. These two structures were used to design our models. A Dynamic Causal Model was used to investigate signal propagation between and within these two regions. After fitting a model to each subject, a random effects analysis demonstrated that red/kitchen input modulated the IFG to CS connection and IFG directly. The study demonstrated overall, that higher contextual details yield worse memory outcomes. The model in which the IFG to CS connection was modulated by Kitchen/Red prompting was most favorable. Importantly, the only connectivity effect that correlated with behavior was the strength of the self-inhibitory IFG to IFG connection, which was significantly higher in young participants ($P = 0.0017$) and correlated with higher recall performance. The decreased inhibition of the IFG to IFG intrinsic connection support previous conclusions that older brains experience a posterior to anterior shift.

Disclosures: **S. Punzell:** None. **E. Dowlati:** None. **S. Adams:** None. **R. Moran:** None.

Nanosymposium

679. Human Cognition: White Matter and Functional Connectivity

Location: 146C

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 679.09

Topic: F.01. Human Cognition and Behavior

Support: MUSC-Radiology Pilot Project

Title: Diffusional kurtosis imaging assessment of brain plasticity in cognitively intact older adults

Authors: L. D. HEWETT[1], M. V. SPAMPINATO[2], A. BENITEZ[2], A. TABESH[2], C. CHAN[2], R. DEARDORFF[2], H. COLLINS[2], *M. FALANGOLA[2];
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Abstract: Several studies have demonstrated the ability of diffusion MRI to identify brain microstructural changes, yielding potential biomarkers of brain plasticity due to cognitive interventions designed to maintain cognitive health and attenuate age-related decline. Thus, the purpose of this study was to investigate if diffusional kurtosis imaging (DKI) could detect brain plasticity following short-term web-based cognitive training in cognitively intact older adults. DKI is a minimal extension of diffusion tensor imaging (DTI) that allows for the incorporation of diffusional non-Gaussianity, from which white matter modeling (WMM) metrics such as

myelin integrity and axonal density can be derived. These preliminary data were acquired from 6 right-handed cognitively intact older adults (ages 60-75), with each participant providing data (cognitive assessment and MRI) at baseline (pre-training) and follow-up (at 12 weeks from baseline). Cognitive training consisted of pre-determined modules created by Lumos Labs, Inc. (San Francisco, CA) composed of 108 sessions (over 12 weeks) designed to improve memory, attention, processing speed, problem solving and cognitive flexibility. To evaluate cognitive status at baseline and after training, we used CogState™, a computerized multi-dimensional set of brief cognitive tests designed to be sensitive to change in neurocognitive function. DTI, DKI and WMM metrics quantifying brain microstructural changes were calculated. All subjects' fractional anisotropy (FA) maps were registered to the FA template (FMRIB58_FA) in FSL, and a mean FA skeleton (threshold of 0.2) was created. Region of interest were defined based on the Johns Hopkins University ICBM white matter label atlas [JHU ICBM-DTI-81]. Paired sample t-tests (uncorrected for multiple comparisons) were used to determine pre vs. post treatment changes in diffusion metrics and cognitive test scores. After training, improvements in the CogState™ "Detection Task" measuring processing speed ($p=0.05$) and "One Back Task" measuring working memory ($p=0.01$) were observed. All participants showed Lumosity training improvement in all five areas studied. Increase in diffusion metrics were seen in different WM tracts, including fornix (mean kurtosis ($p<0.03$)), genu of the corpus callosum (radial kurtosis ($p<0.05$)) and right cingulum (mean kurtosis ($p<0.01$) and axonal water fraction (AWF; $p<0.02$).These results, although preliminary, indicate a possible effect of cognitive training on processing speed and memory, with concomitant microstructural changes in brain white matter.

Disclosures: L.D. Hewett: None. **M. Falangola:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Lumos Labs, Inc., San Francisco, CA. **A. Benitez:** None. **A. Tabesh:** None. **C. Chan:** None. **R. Deardorff:** None. **H. Collins:** None. **M.V. Spampinato:** None.

Nanosymposium

679. Human Cognition: White Matter and Functional Connectivity

Location: 146C

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 679.10

Topic: F.01. Human Cognition and Behavior

Support: K01 AG040197

P50 AG005134

P41 RR14075

P01 AG036694

R01 AG034556

R01 AG046396

Marie Curie IOF

Title: fMRI functional connectivity between the caudate and large-scale cortical networks in aging: Relations to dopamine PET

Authors: *A. RIECKMANN[1,2], K. R. A. VAN DIJK[1,3], R. A. SPERLING[1,4,8], K. A. JOHNSON[5,4,6,8], R. L. BUCKNER[1,3,5,7], T. HEDDEN[1,5];
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Abstract: The caudate is functionally coupled with the fronto-parietal control network and the default network (Choi et al. 2012, J Neurophys). Studies have shown associations between cortico-striatal resting state connectivity and pharmacological manipulations of the dopamine system in young adults (e.g. Cole et al. 2012, Cereb Cortex). Here, we explore effects of age on caudate connectivity with the fronto-parietal network and the default network and whether a PET-based measure of the dopamine system is related to altered caudate functional connectivity in aging. fMRI resting-state functional connectivity between caudate and the fronto-parietal network, the default network and the dorsal attention network was computed for 53 older (66-86 years) and 42 younger (18-32 years) adults. The dorsal attention network was included as a control network where we expected no positive associations with caudate. Older adults also underwent a PET scan to determine striatal dopamine transporter density. Consistent with the functional parcellation in younger adults by Choi et al., the caudate was positively coupled to the fronto-parietal network and, to a lesser degree, to the default network in both age groups. The dorsal attention network was not positively coupled to the caudate. In the older adults, reduced caudate connectivity with the fronto-parietal network was related to lower striatal dopamine transporter density ($r = .27$, $p < .05$), suggesting that age-related decline in striatal functional connectivity may, in part, be explained by decline in markers of the dopamine system. However, no significant associations were found between caudate-default network connectivity and dopamine transporter density and when comparing functional connectivity across age groups, a significant interaction was found between age group and network. Whereas caudate connectivity with the fronto-parietal network was significantly reduced in older adults, caudate connectivity with the default network was significantly increased. This pattern was replicated in an

independent sample of 108 young subjects and 177 old adults, and not readily explained by group differences in motion or scan quality. That said, differences in brain morphometry between groups may introduce subtle, but consistent, differences in multi-modal registration and normalization of subcortical structures. This may lead to systematic distortions in the anatomical boundaries of the caudate, biasing the region of interest in older adults towards subfields of the caudate coupled to the default network. Further exploration of age-related changes in connectivity with subfields of the striatum is warranted.

Disclosures: **A. Rieckmann:** None. **K.R.A. Van Dijk:** None. **R.A. Sperling:** None. **K.A. Johnson:** None. **R.L. Buckner:** None. **T. Hedden:** None.

Nanosymposium

679. Human Cognition: White Matter and Functional Connectivity

Location: 146C

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 679.11

Topic: F.01. Human Cognition and Behavior

Support: Canadian Institutes of Health Research (CIHR)

Title: Selective effects of aging on hippocampal subfields: A high-field magnetic resonance imaging study

Authors: ***N. V. MALYKHIN**[1,2], Y. HUANG[1], F. OLSEN[1], P. SERES[1], R. CARTER[1]; [1]Biomed. Engin., [2]Ctr. for Neurosci., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Objectives: Understanding brain changes associated with normal aging is fundamentally important and critical to understanding age-related neuropsychiatric disorders. Given that memory declines with age and the hippocampus is the most important memory structure, the hippocampus might be particularly vulnerable to effects of aging. The hippocampal formation itself consists of several histologically-defined subfields, including the dentate gyrus, the cornu ammonis, and the subiculum. Although aggregate hippocampal volume reduction in normal aging has been confirmed in many magnetic resonance imaging (MRI) studies, the detailed underlying structural alterations contributing to the overall volume reduction have yet to be specified. It is possible that age- related shrinkage is more evident in some subfields of the hippocampal formation or subregions. The aim of this study was to investigate the effects of normal aging on hippocampal subfields using high-field MRI. Methods: A total of 140 healthy volunteers (18-85 years old) were recruited for this study. Images were acquired using a T2-

weighted 2D FSE and a T1-weighted 3D MPRAGE sequences on a Varian 4.7T scanner. The hippocampal subfields were manually traced with the DISPLAY software using reliable volumetric protocol developed by our group. All volumetric measurements were adjusted to individual intracranial volumes. Pearson's correlation coefficient was used to determine the relationship between age and hippocampal subfield volumes. Results: We found significant negative effects of aging on global volume of the hippocampus (left: r= -.283, p=0.001; right: r=-.261, p=0.002) and volume of the hippocampal body (left: r= -.368, p<0.001; right: r=-.382, p<0.001). Volumes of the hippocampal head and tail were not significantly affected by age (all p>0.05). Age negatively correlated with total volume of the dentate gyrus (left: r= -.238, p=0.005; right: r=-.239, p=0.005) cornu ammonis (left: r= -.227, p=0.007; right: r=-.168, p=0.048) and subiculum (left: r= -.148, p=0.081; right: r=-.194, p=0.021) subfields. However, within the hippocampal subfields only volumes of cornu ammonis within hippocampal body (left: r= -.356, p<0.001; right: r=-.368, p<0.001), dentate gyrus within hippocampal body (left: r= -.376, p<0.001; right: r=-.308, p<0.001) and head (left: r= -.22, p=0.009; right: r=-.222, p=0.008), and subiculum within right hippocampal body (r=-.180, p=0.033) negatively correlated with age. Conclusions: Our findings suggest that dentate gyrus and cornu ammonis subfields of the hippocampus are the most vulnerable to aging process, while subiculum subfield is more preserved.

Disclosures: N.V. Malykhin: None. **Y. Huang:** None. **F. Olsen:** None. **P. Seres:** None. **R. Carter:** None.

Nanosymposium

679. Human Cognition: White Matter and Functional Connectivity

Location: 146C

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 679.12

Topic: F.01. Human Cognition and Behavior

Support: NICHD K12HD051959

NIMH MH090291

Title: The depletion of sex steroid hormones during the menopausal transition is related to altered prefrontal and hippocampal signaling during working memory and memory encoding

Authors: *E. G. JACOBS[1], B. WEISS[3], S. WHITFIELD-GABRIELI[4], A. KLIBANSKI[2], J. M. GOLDSTEIN[2];

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Abstract: A rapidly growing body of work from rodents and nonhuman primates has established estradiol's influence on synaptic organization within the prefrontal cortex (PFC) and hippocampus. Consistent with these findings, previous work from our group (Jacobs J. Neurosci. 2011) demonstrated significant estradiol-dependent effects on DLPFC fMRI BOLD and working memory performance in young women. Given estradiol's regulation of memory circuitry, the loss of ovarian estrogens during menopause likely plays a significant role in shaping age-related neural changes in mid-life. To investigate this, healthy mid-life men and women (N=132; age range 46-53) who are part of a prospective birth cohort study were enrolled in a follow-up fMRI study. Menstrual cycle histories in conjunction with fasting serum samples collected on the morning of the scan day were used to determine pre/peri/post-menopausal status of women per STRAW guidelines. Participants performed a visual working memory task and a verbal memory encoding task during fMRI scanning. Results show robust changes in DLPFC and hippocampal function over the menopausal transition, despite minimal variance in chronological age. During working memory, women exhibited greater DLPFC activity as estradiol levels declined and FSH levels increased. These results are consistent with our previous work in young women, showing less DLPFC activity sustained across working memory blocks during high versus low estradiol conditions (despite indistinguishable performance), a putative marker of neural efficiency. We see a similar inefficient DLPFC response in mid-life as ovarian estrogen levels decline. These data underscore the importance of studying adults early in the aging process in order to understand sex-specific mechanisms that may shape cognitive aging trajectories and, ultimately, disease-risk. Examining the hormonal regulation of memory circuitry within a cognitive neuroscience framework may help resolve key descrepancies between basic animal and clinical research on estrogen therapy.

Disclosures: E.G. Jacobs: None. **B. Weiss:** None. **S. Whitfield-Gabrieli:** None. **A. Klibanski:** None. **J.M. Goldstein:** None.

Nanosymposium

679. Human Cognition: White Matter and Functional Connectivity

Location: 146C

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 679.13

Topic: F.01. Human Cognition and Behavior

Support: NIH NINDS R01 NS075565

NIH NIA F31 AG040890

Title: Obesity-related differences in regional white matter microstructure in midlife

Authors: *A. C. BIRDSILL, M. M. GONZALES, D. E. EAGAN, S. KAUR, W. J. HERTZING, A. P. HALEY;
The Dept. of Psychology, The Univ. of Texas At Austin, Austin, TX

Abstract: The aging US population and the recent rise in the prevalence of obesity are two phenomena that merge in findings that obesity in midlife is a risk factor for dementia, a particularly costly disease, in later life. Recently, obesity, as measured by body mass index, has been associated with white matter microstructural differences as measured by diffusion-tensor imaging (DTI) in a small sample of adults (Verstynen et al., 2012); however, it is unknown if this association exists specifically in midlife. The current study hypothesized a negative relationship between white matter microstructure, as measured by fractional anisotropy (FA) and abdominal obesity, as measured by waist-to-hip ratio (WHR). Healthy participants (N=146) between the ages of 40-61 (M = 49.5) underwent MRI scanning with a 3T Siemens Skyra system (Malvern, PA) and a general health assessment. Eddy current correction and tensor fitting were performed using FSL v5.0 (Oxford, UK). The DTI-TK toolkit (http://dti-tk.sourceforge.net/) was used to register all subjects to a population-specific template. We chose 13 regions of interests from the JHU DTI-based white-matter atlas, based on the previous study by Verstynem et al. and implications for cognitive functioning. The atlas was warped to the study-specific template and FA was calculated in normalized space. Linear regression was used with regional FA as the dependent variable and WHR as the independent variable, controlling for age and sex. FA in four regions: (1) the middle cerebellar peduncle (β = 0.244), (2) the pontine crossing tract (β = 0.329), (3) the splenium of the corpus callosum (β = 0.342), and (4) the bilateral cingulum adjacent to the hippocampus (β = 0.329) were all positively associated with WHR ($p < 0.01$). While the mechanisms behind these unexpected findings are unclear, these results raise the possibility that the relationship between obesity and the brain may fluctuate throughout the lifespan. Reference: Verstynen, T. D., Weinstein, A. M., Schneider, W. W., Jakicic, J. M., Rofey, D. L., & Erickson, K. I. (2012). Increased Body Mass Index Is Associated With a Global and Distributed Decrease in White Matter Microstructural Integrity. Psychosomatic Medicine, 74(7), 682-690. doi:10.1097/PSY.0b013e318261909c

Disclosures: **A.C. Birdsill:** None. **M.M. Gonzales:** None. **D.E. Eagan:** None. **S. Kaur:** None. **W.J. Hertzing:** None. **A.P. Haley:** None.

Nanosymposium

680. Novel Assays: Nanotools for Neuroscience I

Location: 150A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 680.01

Topic: G.04. Physiological Methods

Support: Human Frontier Science Program

Neuroventures

Title: Nanoelectrode-membrane interfaces: Designing a better gigaseal

Authors: *M. R. ANGLE[1,3], Y. KONG[1], A. SHIDHAM[2], A. T. SCHAEFER[3], N. A. MELOSH[1];
[1]Materials Sci. and Engin., [2]Computer Sci., Stanford Univ., Stanford, CA; [3]Behavioural Neurophysiol., Max Planck Inst. for Med. Res., Heidelberg, Germany

Abstract: Despite significant advances in the fabrication of nanoscale devices for electrophysiology, these new tools still lack the utility and robustness of whole-cell patch recording. Specifically, nanofabricated devices rarely achieve intracellular access without simultaneously disrupting the plasma membrane, causing changes in both somatic input resistance and resting membrane potential. Patch pipettes, by contrast, form tightly adherent junctions with the plasma membrane, allowing for local membrane rupture without cellular leakage. Unfortunately, the precise mechanisms of "gigaseal" formation in patch pipettes are not well understood, making the interface difficult to recreate in other systems. Here we describe an alternative approach to interfacing with the plasma membrane. Whereas previous attempts have sought to emulate the patch pipette, focusing on the interactions between the cell membrane surface and surface of the electrode, we have designed probes that integrate into the interior of the lipid bilayer, in the hydrophobic core itself. The approach has two major advantages: (1) interacting directly with the core of the lipid bilayer avoids issues related to the heterogeneity of cell-surface expression and (2) exclusion of water from the inorganic-membrane interface can result in much higher seal resistance per unit length. We will describe ongoing characterization of engineered inorganic-membrane interfaces, both electrical measurements and electron microscopy. Additionally, we will present electrical models to better elucidate the recording capability of different interfaces. Designing, characterizing, and modeling the nanoelectrode-membrane interactions will be a critical step in the design of next-generation tools for neurophysiology.

Disclosures: **M.R. Angle:** None. **Y. Kong:** None. **A. Shidham:** None. **A.T. Schaefer:** None. **N.A. Melosh:** None.

Nanosymposium

680. Novel Assays: Nanotools for Neuroscience I

Location: 150A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 680.02

Topic: G.04. Physiological Methods

Support: Sponsored research grant, Nanovision Biosciences

Title: Algorithms and nanoscale neural stimulation: Physiologic inspired light adaptation of optoelectronic nanowire stimulation of the neural retina

Authors: *G. A. SILVA;
Bioengineering, UCSD, LA JOLLA, CA

Abstract: The goal of electrical stimulation of the neural retina is to restore functional vision by transducing luminous changes into spatial and temporal electrical stimuli capable of providing the brain with as much information as possible about the visual world. While desensitization of ganglion cell responses to electrical stimulation has been shown phenomenologically in vitro and in vivo, little work has systematically characterized retinal adaptation induced by light modulated electrical stimulation across physiologic luminous intensities, and there is no data whatsoever on how the retina adapts to nanoscale electrical stimulation. Regardless of whether electrical adaptation effects are taken into consideration in the design of prosthesis devices or not, it is a fundamental property of the retina that will affect the operation and efficacy of a device. In this work we are beginning to investigate how the inner retina adapts to light modulated electrical stimulation from optoelectronic nanowire electrodes. This technology is a light sensitive neural stimulation nanotechnology composed of arrays of optoelectronic nanowires bundled into small effective electrodes. The eventual goal is to develop a neuromimetic algorithm based on physiological data and theoretical models of biological photoreceptor light adaptation from our prior work, in order to extend the dynamic range of retinal electrical stimulation. In this talk we will introduce the challenge being addressed and discuss our initial conceptual approach to solving it, including a description of our neuromimetic (i.e. neural imitating) algorithm that to a first approximation is designed to adapt the output of the nanowires to the native adaptation that occurs in biological photoreceptors. We will end the talk by providing a ‘to do’ and challenge list that remains to be solved.

Disclosures: G.A. Silva: None.

Nanosymposium

680. Novel Assays: Nanotools for Neuroscience I

Location: 150A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 680.03

Topic: G.04. Physiological Methods

Support: NSF DBI-1055112

Packard Fellowship

NIH 1DP2NS082125

Title: Nanoelectrodes for improved electrophysiology recording

Authors: *B. CUI, Z. LIN, W. ZHAO, Y. CUI, C. XIE;
Stanford Univ., STANFORD, CA

Abstract: The electric coupling between the cell membrane and the measuring electrode is crucial for the electrophysiology performance. Recent studies show that vertical nano- and micro- electrodes protruding from a flat surface significantly enhance signal detection. The high membrane curvature induced by vertical electrodes enhances the membrane-electrode coupling by reducing the gap between the cell membrane and the electrode surface. Here, we develop nanoelectrodes of a new geometry, namely nanotubes. When cells are cultured on nanotube electrodes, the cell membrane not only wraps around the outside of the tubes, but also protrudes deep into the hollow center. This nanotube geometry further enhances the membrane-electrode coupling and results in longer intracellular access and records larger amplitude of action potentials. This study suggests that the nanoelectrode performance can be significantly improved by optimizing the electrode geometry.

Disclosures: B. Cui: None. **Z. Lin:** None. **W. Zhao:** None. **Y. Cui:** None. **C. Xie:** None.

Nanosymposium

680. Novel Assays: Nanotools for Neuroscience I

Location: 150A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 680.04

Topic: G.04. Physiological Methods

Title: Nanowire electrophysiology for high-throughput cell sorting and screening

Authors: *J. T. ROBINSON[1], D. VERCOSA[2], A. M. BELL[2];
[1]Electrical and Computer Engin., [2]Rice Univ., Houston, TX

Abstract: Current methods to record and control the potential across the cell membrane represent an experimental bottleneck for both characterizing ion channel kinetics, and designing proteins for monitoring and manipulating neural activity. For example, conventional patch clamp electrophysiology relies on highly trained researchers to manually align glass micropipettes to individual cells - a process that typically requires tens of minutes to measure from a single cell. Alternative approaches based on planar patch clamp devices and microfluidic pores help to accelerate the measurement process, but cells studied with these devices are sealed to the micron-sized pores, preventing subsequent sorting and genetic profiling. To overcome the limitations of current electrophysiology methods we have developed a device to rapidly control or record the transmembrane potential, and subsequently sort these cells based on their electrophysiological phenotype. We have created this novel electrophysiology-assisted cell sorter by incorporating nanowire electrodes into a microfluidic chip. Nanowires can penetrate the cellular membrane and control or record the transmembrane potential and can easily release the cell into the microfluidic channel where we can then route and sort the cell. Our device represents a major improvement in experimental throughput that will drive the development of new genetic-based approaches to measure and manipulate neural activity including variants of optically- and chemically-gated ion channels and voltage-sensitive proteins.

Disclosures: J.T. Robinson: None. **D. Vercosa:** None. **A.M. Bell:** None.

Nanosymposium

680. Novel Assays: Nanotools for Neuroscience I

Location: 150A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 680.05

Topic: G.04. Physiological Methods

Title: Neural dust: An untethered, distributed ultrasonic backscattering system for scalable and chronic brain-machine interfaces

Authors: *D. SEO, J. CARMENA, J. RABAEY, E. ALON, M. MAHARBIZ;
UC Berkeley, Berkeley, CA

Abstract: A major hurdle in brain-machine interfaces (BMI) is the lack of an implantable neural interface system that remains viable for a substantial fraction of a primate lifetime. Recently, sub-mm implantable, wireless electromagnetic (EM) neural interfaces have been demonstrated in an effort to extend system longevity and enable experiments with freely-behaving subjects. However, it is an often overlooked fact that EM systems do not scale down well in size below the millimeter scale due to the severe inefficiency of coupling radio waves at these scales within tissue. We present an ultra-miniature as well as extremely compliant system that enables massive scaling in the number of neural recordings from the brain while providing a path towards truly chronic BMI. This is achieved via two fundamental technology innovations: 1) 10 - 100 μm scale, free-floating, independent sensor nodes, or *neural dust*, that detect and report local extracellular electrophysiological data via ultrasonic backscatter, and 2) a *sub-cranial mm-scale ultrasonic interrogators* that establish power and communication links with the neural dust. The interrogator array is placed beneath the skull and below the dura mater, to avoid strong attenuation of ultrasound by bone and is powered by an external transceiver via EM energy transfer. In this talk, we will cover key concepts, fundamental system design trade-offs and ultimate size, power, and bandwidth scaling limits of a neural recording platform built from low-power electronics coupled with ultrasonic power delivery and backscatter communication. We will also present a proposed communication scheme for multi-neural dust interrogation and data acquisition using ultrasonic beam-forming with a distributed, ultrasonic interrogator array. Finally, we will discuss ongoing efforts in the demonstration of both acute and chronic *in vivo* recording from the rodent cortext using neural dust.

Disclosures: D. Seo: None. **J. Carmena:** None. **J. Rabaey:** None. **E. Alon:** None. **M. Maharbiz:** None.

Nanosymposium

680. Novel Assays: Nanotools for Neuroscience I

Location: 150A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 680.06

Topic: G.04. Physiological Methods

Support: Mayo-Karolinska Collaborative Travel Award

Mayo-Karolinska Collaborative Grant

The Grainger Foundation

Title: Approaching the artificial synapse: Wirelessly controlled neurotransmitter release and electrochemical sensing system

Authors: *E. K. ROSS[1,2,3], S. LÖFFLER[6], P.-K. MIN[4,2], D. SIMON[7], D. NILSSON[8], K. E. BENNET[5,2], M. BERGGREN[7], A. RICHTER-DAHLFORS[6], K. H. LEE[4,2,3];
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Abstract: Introduction: Specific circuit dysfunction at the neurochemical level has been associated with a variety of neurologic and neuropsychiatric disorders. Recently, region-specific deep brain stimulation (DBS) has successfully been used for these disorders, though the mechanisms remain to be elucidated. Although effective, DBS is not specific in its neuromodulation, whereby effects of electrical stimulation remain hard to control and depend highly on targeting accuracy within neuronal circuits. Here, we investigate the potential for spatiotemporally controlled neurotransmitter delivery, which may be achieved by combining the recently developed Organic Electronic Ion Pump (OEIP) with neurocontrol and neurochemical recording systems, allowing neurotransmitter release and sensing that mimic the brain's inherent synaptic mechanism. Methods: Using this Organic Electronic Ion Pump (OEIP) technology, we used electric current to translate precise, spatiotemporally controlled transport of neurotransmitters. To electrically drive this neurochemical release, we used in-house developed Mayo Investigational Neuromodulation Control System (MINCS). Additionally, to approach the concept of the artificial synapse, the Wireless Instantaneous Neurochemical Concentration Sensor (WINCS) system was used to measure and record this neurotransmitter release. Results: Here, we report the successful combination of these state of the art technologies, and demonstrate wirelessly controlled real-time neurotransmitter release and recording. We found specific neurotransmitter transport with monophasic electrical pulses supplied from the MINCS system and later detected with WINCS. We also show that the waveform of electrical signal with which the OEIP is powered plays a major role for neurotransmitter delivery. Conclusion: We succeeded in demonstrating spatiotemporally controlled neurotransmitter delivery by coupling the MINCS/WINCS systems with the OEIP, and show promising feasibility for the combination. Importantly, these results provide evidence for a biomimetic neurotransmitter delivery machine-to-cell interface based solely on wirelessly controlled neurochemical release and sensing.

Disclosures: **E.K. Ross:** None. **S. Löffler:** None. **P. Min:** None. **D. Simon:** A. Employment/Salary (full or part-time):; OBOE IPR. **D. Nilsson:** A. Employment/Salary (full or

part-time):; OBOE IPR. **K.E. Bennet:** None. **M. Berggren:** A. Employment/Salary (full or part-time):; OBOE ICT. **A. Richter-Dahlfors:** None. **K.H. Lee:** None.

Nanosymposium

680. Novel Assays: Nanotools for Neuroscience I

Location: 150A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 680.07

Topic: G.04. Physiological Methods

Title: Manipulating neuronal differentiation and growth via magnetic nanoparticles and nanoparticle-NGF complex

Authors: *O. SHEFI[1], N. ALON[1], M. MARCUS[1], T. HAVDALA[2], H. SKAAT[3], M. KARNI[1], M. PASSIG-ANTMAN[1], S. MARGEL[3], A. SHARONI[2];
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[2]Dept. of Physics and Inst. of Nanotechnologies and Advanced Materials, Ramat Gan, Israel;
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Abstract: The ability to manipulate neuronal organization and growth has extensive implications in neuronal regeneration and tissue engineering. It has been shown that physical forces play a key role in shaping neuronal structure and in interactions between neurons and their vicinity. In the present study we use magnetic nanoparticles (maghemite) as mediators to apply physical forces locally and as carriers of neuronal growth factors. We use these nano complexes in order to locate cells, promote neuronal growth and affect growth orientation. We designed and generated magnetic fields with controlled magnetic flux densities at multiple scales of size and strength. In addition to strong permanent bar magnets and electromagnets we fabricated a unique device, embedded with micropatterned ferromagnets that can be magnetized selectively. First, we incubated, prior to plating, the treated cells, PC12 cells and primary neurons, in medium enriched with iron oxide nanoparticles conjugated to fluorescent tag. Both types of cells uptake the nanoparticels (of 100nm) and turned sensitive to the magnetic stimulation with no cytotoxic effect. We successfully improved the cells motility and attracted the cells to one magnetic pole or the other or towards magnetic 'hot spots'. Plating PC12 cells atop the micropatterned device has led to an organized network of clusters of cells. After cells adhered to the plate the magnetic field affected the neuronal outgrowth orientation, combining the normal chemical signaling with the applied physical forces. In addition, we found that covalent conjugation of the magnetic nanoparticles to Nerve Growth Factor (β-NGF) which is a critical component in nerve tissue

development and repair enhanced the typical effect of NGF. Treatment with the nanoparticle-NGF complex has led to a promoted differentiation and to more complex dendritic trees. Even low doses were sufficient to trigger the promoted differentiation process. Following incubation, single cell imaging revealed particles accumulation in the soma (but not in the nucleus), in the growth cones and at branching points. Currently we use the controlled magnetic fields to direct the NGF-nano-based complexes towards specific target sites for local triggering, thus, to regulate the differentiation and network formation. Our study presents an emerging magneto-chemical method for the manipulation of neuronal growth opening new directions in non-invasive neuronal repair.

Disclosures: O. Shefi: None. **N. Alon:** None. **M. Marcus:** None. **M. Passig-Antman:** None. **M. Karni:** None. **T. Havdala:** None. **A. Sharoni:** None. **H. Skaat:** None. **S. Margel:** None.

Nanosymposium

680. Novel Assays: Nanotools for Neuroscience I

Location: 150A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 680.08

Topic: G.04. Physiological Methods

Support: Human Frontier Science Program

Medical Research Council

Max-Planck-Gesellschaft

University of Heidelberg

Title: A scalable design for neuronal recordings *in vivo* using readout integrated circuits and cast microwire bundles

Authors: *A. T. SCHAEFER[1,2], M. ANGLE[3], W. WRAY[4], R. RACZ[5], N. KISKIN[5], M. KOLLO[5];
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Abstract: In most areas of the mammalian brain, functionally coherent networks consist of thousands to millions of neurons. The distributed nature of neuronal coding within these networks and the reliance of these codes on relative spike timing mean that correlating network activity to complex patterns of behaviour requires that a substantial fraction of the neurons within the network be recorded simultaneously. In addition, short distance connections are very important in neuronal circuits, and unambiguous source attribution within these local assemblies requires that sampling be not only broad but also dense. An ideal electrophysiological recording technique therefore should employ small electrodes in a highly scalable configuration, in order to record from many single neurons. Finally, to avoid the signal-to-noise problems often associated with small electrodes, each electrode should also be optimized for both low electrode-electrolyte interfacial impedance and low stray capacitance. Here we present a novel approach that provides a solution to this challenge by combining bundles of insulated metal wires with arrays of highly sensitive amplifiers based on readout integrated circuits (ROICs) from high-speed infrared cameras. Glass-ensheathed metal wires with customizable metal core (2-15 um) and glass (10-40 um) diameters were produced by means of the Taylor-Ulitovsky method, and assembled in bundles with 100-100.000 individual wires using a custom-designed semi-automated process. Individual electrodes had a stray capacitance as low as 0.5 pF/mm, while electrode impedance (1 kHz) was reduced below 30 MΩ by electrochemical Iridium Oxide deposition. The readout of recorded currents was established by using a ROIC of a Xenics Cheetah 640-CL1700 camera, incorporating over 300,000 capacitive transimpedance amplifier circuits with <10fF feedback capacitance at a pixel pitch size of 20 um. Tight but reversible coupling between wire bundle and ROIC pixels was assured using a custom-designed parallel force application system. Noise levels were less than 1% with 14bit full range digitisation and 1.6 kHz full frame (640x512 pixel) readout. Our data suggests that the combination of bundles of insulated metal wires and high-speed ROICs provides a highly scalable approach to neuronal unit recordings.

Disclosures: **A.T. Schaefer:** None. **M. Angle:** None. **W. Wray:** None. **R. Racz:** None. **N. Kiskin:** None. **M. Kollo:** None.

Nanosymposium

680. Novel Assays: Nanotools for Neuroscience I

Location: 150A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 680.09

Topic: G.04. Physiological Methods

Title: A transparent organic transistor for electrophysiological stimulation and recording of primary neurons

Authors: *A. PISTONE[1], V. BENFENATI[1], S. TOFFANIN[2], S. BONETTI[2], A. SAGNELLA[3], S. KARGES[2], G. TURATTI[4], M. CHIAPPALONE[5], A. STEFANI[4], G. GENERALI[4], G. RUANI[2], D. SAGUATTI[2], R. ZAMBONI[1], M. MUCCINI[2];
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Abstract: Advanced biomedical tools and device to enable real-time recording and manipulation of communication signals between neural cells are demanded to improve our understanding and controlling mechanism underpinning physiology and pathophysiology of Nervous System. Organic field effect devices based on organic semiconductor materials could be promising alternative over traditional silicon-based ones, by means of improved long-term biocompatibility, mechanical flexibility, adaptable form factor and low cost fabrication. Here, by optical and confocal microscopy, we show that primary sensory Dorsal Root Ganglion (DRG) neurons from post-natal rat can adhere, grow and differentiate on organic semiconductor material interface. Whole-cell patch-clamp experiments demonstrate that electrophysiological properties of neurons are not altered by growth on organic perylene type materials and they maintain their firing properties even after a prolonged time of cell-culturing. By coupling device to patch-clamp we show that Organic Cell Stimulating and Sensing Transistors (O-CSTs) enables depolarization and hyperpolarization of primary neurons membrane potential. Comparative extracellular recording performed on the same neuronal preparation by O-CST and by micro electrode array (MEA) demonstrate that the O-CST device enable recording from neurons with maximal amplitude-to-noise ratio 16 times better than MEA. A critical point in the neural engineering and neuroprosthesis regards the inflammatory gliotic reactions in the area surrounding the implant with glial scar formation, which alter the device performance over long term. We prove also the biocompatibility of the organic interface with the primary cortical astrocytes. Viability, electrophysiological behavior and levels of glial fibrillar acid protein (GFAP) expression are evaluated. The possibility to exploit transparency of O-CST to study astroglial cell calcium signaling is also explored. Collectively our results indicate that O-CST provides stimulation, manipulation and recording of primary neurons, paving the way to a new generation of devices for investigation of electrophysiological properties of neural cell and for future neuroprosthesis.

Disclosures: **A. Pistone:** None. **V. Benfenati:** None. **S. Toffanin:** None. **S. Bonetti:** None. **A. Sagnella:** None. **S. Karges:** None. **G. Turatti:** None. **M. Chiappalone:** None. **A. Stefani:** None. **G. Generali:** None. **G. Ruani:** None. **D. Saguatti:** None. **R. Zamboni:** None. **M. Muccini:** None.

Nanosymposium

680. Novel Assays: Nanotools for Neuroscience I

Location: 150A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 680.10

Topic: G.04. Physiological Methods

Support: Stanford Neuroventures

NSF CPN

Title: Nano-rings for multifunctional neuronal interfaces

Authors: *N. MELOSH, N. HOHMAN, K. CHANG, T. BOZORG-GRAYELI;
Materials Sci., Stanford Univ., Stanford, CA

Abstract: Engineered nanostructures offer the ability to rationally design interfaces to cells and neurons that may overcome many of the challenges facing electrical interfaces to neurons, both in vitro and in vivo. These include high seal resistances, single cell specificity, and scalable arrays on chip. Here we describe "NanoRings" as a general architecture for multi-functional interfaces to single cells. These make intimate contact with the cell with sufficient internal space to embed multiple devices within the ring, providing single-cell specificity and a controlled environment for active devices. We will describe fabrication and testing of this general design concept, and show how it can be flexibly redesigned for particular applications. Both electrical and chemical interfaces to single cells are possible for long term, multifunctional monitoring of single cells. These platforms are being extended for in vivo and slice testing, where the single cell specificity and immunity to micromotions are particularly beneficial.

Disclosures: **N. Melosh:** None. **N. Hohman:** None. **K. Chang:** None. **T. Bozorg-Grayeli:** None.

Nanosymposium

680. Novel Assays: Nanotools for Neuroscience I

Location: 150A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 680.11

Topic: G.04. Physiological Methods

Title: CMOS-nanowire electrode array for high-fidelity multiplexed interrogation of neuronal circuits

Authors: *H. PARK;
Dept. of Chem. and Chem. Biol., Harvard Univ., CAMBRIDGE, MA

Abstract: Deciphering the rules by which neuronal circuits store and process information requires not only the structural determination of the physical wiring diagram of neurons but also the measurement of functional connectivity between them. Recently, using vertical silicon nanowires (NWs) as intracellular probes, we demonstrated a highly scalable electrode platform that has the potential for meeting this critical need. In this presentation, I will describe our new efforts to transform this passive prototype into a fully integrated, active CMOS-nanoelectrode array (CNEA) with significantly improved capability. These CNEA will enable massively parallel, single-cell-resolution recording/stimulation of a large number of neurons, thereby enabling the functional mapping of a complex neuronal network.

Disclosures: H. Park: None.

Nanosymposium

680. Novel Assays: Nanotools for Neuroscience I

Location: 150A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 680.12

Topic: G.04. Physiological Methods

Title: Silk fibroin substrates as neural, instructive interface that guides growth, differentiation and functional properties of primary neurons and astrocytes *in vitro*

Authors: A. SAGNELLA[1], A. PISTONE[2], T. POSATI[1], S. BONETTI[3], C. DIONIGI[3], C. CHIECO[4], S. TOFFANIN[3], G. RUANI[3], R. ZAMBONI[2], M. SPIRA[5], M. MUCCINI[3], *V. BENFENATI[2];
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Abstract: Biomaterials that enable the control of functional properties in neural cells have major potential for their use in neural tissue engineering, targeted drug release or stem-cell-based neuroregenerative medicine. In this rapidly emerging area, particular attention is devoted to the engineering and use of biomaterials that could be integrated in biocompatible electronic devices for in vitro or in vivo diagnostic as well as therapeutic purposes. Recent research on silk fibroin (SF) from the silkworm Bombyx mori highlights its potential as a sustainable, biocompatible, biodegradable and technological material. However, achieving a thorough biocompatibility can be challenging due to the complex nature of the neural cell response to interactions with biomaterials. Our previous works [1,2] demonstrated the suitability of SF based biomaterials to support the in vitro studies on neurons and astrocytes. In this study single walled carbon nanotubes/silk-fibroin 3D bionanocomposite (3D SWCNT/SF), gold mushroom-shaped microelectrodes (gMuE) - coated substrates and patterned SF film are investigated as SF-based neural engineered interfaces for bioelectronics device intended for electrophysiological investigation of neural cells. Fabrication methods are reported. Chemophysical properties such as conductivity, roughness, optical transparency of SF based interface are studied. Primary cultures of rat dorsal root ganglion (DRG) neurons and cortical astrocytes have been plated on SF based substrates. Morphometric analyses of cells adhesion and growth on silk substrates have been performed by optical, confocal and electron microscopy. To monitor the effect of SF substrates on electrophysiological properties of primary neurons and astrocytes, functional analyses by means of patch-clamp and calcium imaging are performed. Collectively our results demonstrated the promising properties of silk fibroin substrates as neural interface in device and scaffold intended for neuroscience fundamental electrophysiological investigations as well as for neural engineering and regenerative medicine. [1] Benfenati V. et al, Biomaterials 2010, 31, 7883-7891. [2] Benfenati V. et al., Adv. Fun. Mater. 2012, 22, 1-14.

Disclosures: **A. Sagnella:** None. **A. Pistone:** None. **T. Posati:** None. **S. Bonetti:** None. **C. Dionigi:** None. **C. Chieco:** None. **S. Toffanin:** None. **G. Ruani:** None. **R. Zamboni:** None. **M. Spira:** None. **M. Muccini:** None. **V. Benfenati:** None.

Nanosymposium

680. Novel Assays: Nanotools for Neuroscience I

Location: 150A

Time: Wednesday, November 19, 2014, 8:00 AM - 11:15 AM

Presentation Number: 680.13

Topic: G.04. Physiological Methods

Title: An architecture for neural recording using optical time-domain reflectometry

Authors: A. MARBLESTONE[1], D. AMODEI[3], G. CHURCH[4], L. WOOD[5], *E. S. BOYDEN[2]; [1]MIT, Cambridge, MA; [2]MIT, CAMBRIDGE, MA; [3]Stanford Univ., Stanford, CA; [4]Harvard Univ., Boston, MA; [5]N/A, N/A, CA

Abstract: We study the theoretical properties of a potential "hybrid" optoelectronic architecture for performing whole brain activity mapping. We contemplate laying optical fibers bearing periodically spaced activity sensors throughout the entirety of a mammalian brain, and continuously monitoring signals elicited from these sensors by using time-delay reflectometry of optical pulses sent along the fibers. The idea is to sense neuronal activations electrically, acoustically or magnetically from a short standoff (maximizing SNR and removing a need for genetic manipulation), but to probe the apparatus optically (maximizing bandwidth). Optical fibers are used to contain optical probe pulses, such that photons largely remain inside the fibers and do not enter the brain tissue itself, minimizing tissue heating. Interaction of nanoprobe sensors attached to the fiber walls with the evanescent field of the probe pulse leads to activity-dependent changes in the reflection coefficient near the sensor site. This architecture can also be operated in reverse to allow multiplexed optically-driven electrical stimulation. Calculations and simulations define the required parameters of the sensing and readout systems, leading to proposed fabrication and delivery pathways.

Disclosures: A. Marblestone: None. **D. Amodei:** None. **G. Church:** None. **L. Wood:** None. **E.S. Boyden:** None.

Nanosymposium

767. Synaptic Plasticity: Molecular and Circuit Mechanisms

Location: 147A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 767.01

Topic: B.08. Synaptic Plasticity

Support: Z01-ES100221

Title: Neuronal activity induces transcription of two sub-classes of immediate early genes

Authors: P. C. ROMEO[1], M. K. WURZELMANN[1], E. R. BAILEY[1], R. N. SAHA[2], *S. M. DUDEK[1];

[1]Lab. of Neurobio., Natl. Inst. of Envrn. Hlth. Sciences, NIH, Research Triangle Park, NC; [2]Sch. of Natural Sci., Univ. of California, Merced, Merced, CA

Abstract: The brain's ability to learn and form long lasting memories depends on *de novo* gene transcription within a short period of time after environmental stimulation. During this short time period, several genes are transcribed, many of which are immediate early genes (IEGs). In cultures of primary cortical rat neurons we have observed that, similar to what is seen in behaving animals, some IEGs such as activity-regulated cytoskeleton-associated protein (*Arc)* are up-regulated within 5 minutes of stimulation. We have noted that these rapidly responding genes have RNA Polymerase II (Pol II) bound near their transcription start site (TSS) and now define them as Rapid IEGs (Saha *et al.* 2011). Conversely, we have defined Delayed IEGs, such as brain-derived neurotrophic Factor (*Bdnf*), respond more slowly and generally lack a stalled polymerase near their TSSs. Here we present evidence that these IEG subclasses can be further differentiated by their induction requirements. Previous studies have shown that several (Rapid) IEGs can be induced in rats *in vivo* with as little as one lap around a track (Miyashita, *et al*. 2009). Therefore we tested whether Rapid and Delayed IEGs responded differentially to very brief periods of increased neuronal activity. We find that Rapid IEGs, but not Delayed IEGs, are transcribed in response to 2 or 5 minutes of neuronal activity; Delayed IEGs required much longer periods of activity. Moreover, Rapid, but not Delayed IEGs can be induced with pharmacological stimulation of two upstream activators of the MEK/ERK pathway (phorbol 12-myristate 13-acetate (PMA) and forskolin, through PKC and PKA respectively) in the absence of neuronal activity. Inhibitors of the MEK/ERK pathway block the induction of both IEG subclasses. These results suggest that Delayed IEGs require signaling factors in addition to the MEK/ERK pathway, but that ERK activity by itself may be sufficient for the Rapid IEG response. In summary, we present further evidence that Rapid IEG induction is mechanistically distinct from Delayed IEG induction in response to neuronal activity. This research is supported by the Intramural Research Program of the National Institute of Environmental Health Sciences, NIH.

Disclosures: P.C. Romeo: None. **M.K. Wurzelmann:** None. **E.R. Bailey:** None. **R.N. Saha:** None. **S.M. Dudek:** None.

Nanosymposium

767. Synaptic Plasticity: Molecular and Circuit Mechanisms

Location: 147A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 767.02

Topic: B.08. Synaptic Plasticity

Support: NIH Grant R01 NS073930

NIH Grant R21 NS066235

NIH Grant F31 NS074840

Title: Regulation of spine maturation and spine pruning via dendritically synthesized BDNF and NMDAR activity

Authors: *L. L. OREFICE[1], E. WATERHOUSE[2], B. XU[3];
[1]Dept. of Pharmacol. and Physiol., Georgetown Univ., WASHINGTON, DC; [2]Pharmacol. and Physiol., Georgetown Univ., Washington, DC; [3]Neurosci., The Scripps Res. Inst., Jupiter, FL

Abstract: Dendritic spines are the postsynaptic sites for the vast majority of excitatory synapses. Spines progress into their adult forms through three developmental processes: formation, maturation and pruning. The mechanisms controlling spine maturation and spine pruning remain largely unknown. The gene for brain-derived neurotrophic factor (BDNF) produces transcripts with either a short or long 3′ untranslated region. The short-form transcripts are restricted to cell bodies, whereas the long-form transcripts are also present in dendrites to allow for local translation. Our work has shown that somatically synthesized BDNF stimulates spine formation, while dendritically synthesized BDNF is required for spine maturation and spine pruning. Here we investigate how BDNF synthesized in the soma and dendrites achieves its distinct effects on spine morphogenesis, using cultured rat hippocampal neurons. We found that somatically synthesized BDNF was mostly released as mature BDNF constitutively, and promoted spine formation through the TrkB receptor. To the contrary, we found that NMDAR activity stimulated dendritic synthesis of BDNF, which was largely secreted as proBDNF in an activity-dependent manner. proBDNF acted on the $p75^{NTR}$ receptor to mediate spine pruning through activation of the small GTPase, RhoA. Furthermore, we found that the released proBDNF could also be extracellularly converted to mature BDNF by the tPA/plasmin system. Mature BDNF then acted on the TrkB receptor, which lead to Rac1 activation and subsequently stimulated spine head enlargement. Importantly, NMDAR activity was required for BDNF-induced increases in spine head size, spine pruning, phosphorylation of TrkB, and increases in activated Rac1 and RhoA. These results reveal novel biochemical pathways through which dendritically synthesized BDNF coupled with NMDAR activity governs spine maturation and spine pruning.

Disclosures: L.L. Orefice: None. **B. Xu:** None. **E. Waterhouse:** None.

Nanosymposium

767. Synaptic Plasticity: Molecular and Circuit Mechanisms

Location: 147A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 767.03

Topic: B.08. Synaptic Plasticity

Support: 1F311MH103902

NIH MH098016

Title: Role of the Lin28/let-7 axis in gene expression regulation by BDNF

Authors: A. M. AMEN[1], C. R. RUIZ[1], J. SHI[1], *M. K. MEFFERT[2];
[1]Neurosci., [2]Dept Biol Chem & Neurosci, Johns Hopkins Sch. Med., BALTIMORE, MD

Abstract: Stimulus-induced gene expression is essential for imparting endurance to the plastic changes in synaptic networks that underlie learning and are dysregulated in numerous cognitive disorders. In recent years, high-throughput studies have highlighted the importance of post-transcriptional mechanisms in achieving the appropriate stimulus-specificity in gene expression. Brain-derived neurotrophic factor (BDNF) is a widely investigated growth factor that undergoes activity-dependent release and induces synthesis of a highly selective suite of proteins that collectively enhance neuronal growth and excitatory synaptic function. BDNF promotes learning and memory and can induce long-term changes in synaptic plasticity, and misregulation of BDNF signaling is implicated in a range of neurologic diseases and disorders. Our lab has demonstrated that BDNF achieves its characteristic high level of gene-target specificity in neuronal protein synthesis by regulating microRNA (miRNA) biogenesis. MiRNAs are short, single stranded RNA molecules that bind and cause translational suppression of their target mRNAs. We found that BDNF 1) rapidly elevates both Dicer and TRBP proteins, which, as a complex, promote processing of precursor miRNAs into mature miRNAs, and 2) induces Lin28a, an RNA-binding protein associated with pluripotency that blocks production of a subset of precursor miRNAs (primarily Let-7 family miRNAs). Thus, BDNF generally enhances miRNA-mediated repression in neurons by elevating Dicer and TRBP proteins, but alleviates repression specifically of Let-7 miRNA targets, many of which encode pro-growth proteins crucial for synaptic function and plasticity. Lin28a was previously thought to be absent from differentiated cells, and aberrant expression of Lin28a can promote tumorigenesis. As our work is the first demonstration of stimulus-responsive induction and normal physiological Lin28a function in a mature cell type (hippocampal neurons), elucidating the mechanism by which BDNF can induce expression of Lin28a is an important focus of ongoing investigations. We find that Lin28a protein undergoes rapid transcription-independent induction by BDNF, and that this rapid elevation lies downstream of a BDNF-induced phosphorylation event. Additionally, our studies indicate that BDNF differentially regulates Lin28a and its homolog Lin28b, which were

previously thought to be functionally redundant. Ongoing and future experiments aim to further elucidate the molecular mechanisms of Lin28a induction by BDNF, which may provide therapeutic targets for diseases and disorders associated with aberrant BDNF signaling.

Disclosures: **A.M. Amen:** None. **C.R. Ruiz:** None. **J. Shi:** None. **M.K. Meffert:** None.

Nanosymposium

767. Synaptic Plasticity: Molecular and Circuit Mechanisms

Location: 147A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 767.04

Topic: B.08. Synaptic Plasticity

Support: Israel Science Foundation Grant 393/12

Israel Centers of Research Excellence Grant 1796/12

German-Israel Foundation Grant 2299-2291.1/2011

EU Marie Curie Career Integration Grant 618201

NARSAD Young Investigator Award 18795

Title: Transcription networks provide a window into the neural circuitry of addiction

Authors: *A. CITRI;
Safra Ctr. for Brain Sci. & Life Sci. Inst., The Hebrew Univ., Jerusalem, Israel

Abstract: We are fortunate to be active in an extremely exciting period in neuroscience research. This is a period of revolution in our capacity to translate molecular knowledge into novel tools for detailed investigation of neural circuits. The interest of my research group is in how the nervous system encodes experience and modifies future behavior. This “Experience-Dependent Plasticity” is a fundamental feature of the brain, underlying the capacity to develop adaptive behaviors. Our main model system addresses the development of the response to cocaine experience. We apply a uniquely multidisciplinary approach, based primarily upon information acquired from studying the principles of dynamic gene regulation in the nucleus accumbens following cocaine experience in young adult male mice. Building on transcriptional knowledge, we develop tools to investigate the organization and plasticity of the gene regulatory networks encoding cocaine experience. We also develop genetic tools to identify and probe neural

ensembles responsive to cocaine experience, as well as brain regions previously not associated with the reward circuitry. The neural circuit organization and plasticity are probed with an assortment of viral tools (AAV and pseudotyped-Rabies virus), as well as targeted whole-cell patch-clamp electrophysiological recordings. It is our conviction that a multidisciplinary approach will provide substantial insight into the mechanisms of plasticity underlying the establishment of compulsive behavior during the development of addiction to drugs of abuse. Exemplifying our approach, I will present work in which we utilize analysis of transcriptional dynamics following cocaine experience to identify robust rewiring of transcriptional regulation in the nucleus accumbens following abstinence from chronic cocaine exposure. We further identify the mechanisms responsible for the transcriptional plasticity, and the impact of the transcriptional rewiring on the development of the behavioral response to cocaine experience. In the context of identifying novel neural circuits, I will also describe our use of mouse genetics and stereotactic viral manipulations in order to identify and define a neuronal ensemble within the nucleus accumbens responsive to cocaine experience. Detailed whole-cell electrophysiological recordings identify specific changes in synaptic input onto this neuronal neuronal ensemble, while conditional, selective inhibition of this neuronal ensemble demonstrates its function in the development of behavioral sensitization to cocaine.

Disclosures: A. Citri: None.

Nanosymposium

767. Synaptic Plasticity: Molecular and Circuit Mechanisms

Location: 147A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 767.05

Topic: F.02. Animal Cognition and Behavior

Support: 5R01GM054508

Title: WT1-mediated suppression of synaptic plasticity preserves long-term memory encoding in hippocampus

Authors: *C. MARIOTTINI[1], L. MUNARI[1], J. SECO[1], E. GUNZEL[1], S. STERN[3], V. GAO[3], J. HANSEN[1], G. HODES[2], S. RUSSO[2], V. HUFF[4], C. ALBERINI[3], R. BLITZER[1], R. IYENGAR[1];
[1]Pharmacol. and Systems Therapeut., [2]Dept. of Neurosci., Mount Sinai Sch. of Med., New York, NY; [3]Ctr. for Neural Sci., New York Univ., New York, NY; [4]Dept. of Genet., MD Anderson Cancer Ctr., Houston, TX

Abstract: Long-term memory (LTM) relies on input-specific synaptic plasticity, including long-term potentiation (LTP). However, stimuli that are strong enough to induce persistent LTP also engage pro-plasticity mechanisms that extend over large regions of the neuron and can last for hours. Given the temporal constraints on associative learning, there should be some mechanism that deters non-specific learning following a strong event. To identify molecules that mediate this active suppression, we induced LTP in rat hippocampal slices, and used a protein-DNA binding array to screen for activated transcription factors (TFs). Among activated TFs was Wilm's Tumor 1 (WT1), mostly known as a transcriptional repressor and also involved in mRNA processing. Expression of WT1 increased in dorsal hippocampus after LTP induction, and also after inhibitory avoidance training in rats. We ablated hippocampal WT1 activity in rats by injection of WT1 antisense, and in transgenic mice expressing non-functional WT1. Both approaches enabled weak HFS to induce persistent LTP, suggesting that WT1 acts as a plasticity suppressor. In agreement, loss of WT1 function in rats and mice enhanced LTM in two different weak training protocols, contextual fear conditioning (CFC) and novel object placement (NOP). Importantly, in a two-task sequential learning protocol (weak NOP training, followed 48 h later by CFC training), transgenic mice showed normal LTM for NOP but impaired LTM for the subsequent CFC. These results support the hypothesis that WT1, by actively suppressing plasticity for a time after learning, preserves the ability of hippocampal neurons to repeatedly encode LTM for discrete, unrelated tasks. We identified relevant genes under the control of WT1 with mRNA-seq and RT-PCR in rat hippocampus following WT1 knockdown. Regulated genes included members of the chemokine and interleukin superfamilies, as well as IGF2, a known target of WT1. Antagonists of the CCL2 and IGF2 receptors prevented WT1 knockdown from enhancing LTP. Moreover, treatment of WT1 transgenic mice with a CCL2 receptor antagonist before training suppressed the LTM enhancement in the NOP test. These results show that WT1 controls the synthesis of multiple diffusible signaling molecules, which may modulate synaptic plasticity at the circuit level. Taken together, our data indicate that the ability of the hippocampus to repeatedly encode new memories requires a period of plasticity suppression that is transcriptionally regulated, and that WT1 plays an important role in this systems-level function. Such complex balancing of pro- and anti-plasticity mechanisms could play a role in learning disorders including autism.

Disclosures: **C. Mariottini:** None. **L. Munari:** None. **J. Seco:** None. **E. Gunzel:** None. **S. Stern:** None. **V. Gao:** None. **J. Hansen:** None. **G. Hodes:** None. **S. Russo:** None. **V. Huff:** None. **C. Alberini:** None. **R. Blitzer:** None. **R. Iyengar:** None.

Nanosymposium

767. Synaptic Plasticity: Molecular and Circuit Mechanisms

Location: 147A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 767.06

Topic: B.08. Synaptic Plasticity

Support: Revalesio corporation

Title: RNS60, saline containing charge stabilized oxygen based nanostructures, enhances neuronal plasticity via activation of phosphatidylinositol-3 kinase pathway

Authors: A. ROY[1], K. K. MODI[1], S. KHASNAVIS[1], S. GHOSH[2], R. WATSON[2], *K. PAHAN[1,2];
[1]Dept Neurolog. Sci., Rush Univ. Med. Ctr., CHICAGO, IL; [2]Revalesio Corp., Tacoma, WA

Abstract: Increased density of dendritic spines and enhanced synaptic transmission through ionotropic glutamate receptors are important events leading to synaptic plasticity and eventually hippocampus-dependent spatial learning and memory formation. Here we describe an innovative approach to upregulate hippocampal plasticity. RNS60 is a 0.9% saline solution containing charge-stabilized nanostructures that are generated by subjecting normal saline to Taylor-Couette-Poiseuille (TCP) flow under elevated oxygen pressure. RNS60, but not NS (normal saline), PNS60 (saline containing comparable level of oxygen without the TCP modification), or RNS10.3 (TCP-modified normal saline without excess oxygen), stimulated morphological plasticity and synaptic transmission via NMDA- and AMPA-sensitive calcium influx in cultured mouse hippocampal neurons. Using mRNA-based targeted gene array, real-time PCR, immunoblot, and immunofluorescence analyses, we further demonstrate that RNS60 stimulated the expression of many plasticity-associated genes in cultured hippocampal neurons. Activation of type IA, but not type IB, phosphatidylinositol-3 (PI-3) kinase by RNS60 together with abrogation of RNS60-mediated upregulation of plasticity-related proteins (NR2A and GluR1) and increase in spine density, neuronal size, and calcium influx by LY294002, a specific inhibitor of PI-3 kinase, suggest that RNS60 upregulates hippocampal plasticity via activation of PI-3 kinase. Finally, in the 5XFAD transgenic model of AD, RNS60 treatment upregulated expression of plasticity-related proteins PSD95 and NR2A and increased AMPA- and NMDA-dependent hippocampal calcium influx. These results describe a novel mode for stimulating hippocampal plasticity, which may be instrumental in exploring a new therapy for AD and other dementias.

Disclosures: **A. Roy:** None. **K.K. Modi:** None. **S. Khasnavis:** None. **S. Ghosh:** None. **R. Watson:** None. **K. Pahan:** None.

Nanosymposium

767. Synaptic Plasticity: Molecular and Circuit Mechanisms

Location: 147A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 767.07

Topic: F.02. Animal Cognition and Behavior

Support: NIH COBRE 1P20GM103653 - 01A1

Title: Spatial working memory deficits accompany reductions in hippocampal-prefrontal synchrony following inactivation of the ventral midline thalamic reuniens and rhomboid nuclei

Authors: *H. L. HALLOCK, A. L. GRIFFIN;
Psychology, Univ. of Delaware, Newark, DE

Abstract: A growing body of evidence suggests that different types of learning and memory processes are distributed across specialized neural circuits consisting of two or more anatomically- and functionally-connected brain areas. One such neural circuit consists of the dorsal hippocampus (dHC) and the medial prefrontal cortex (mPFC). This circuit is thought to be critically important for spatial working memory (the ability to flexibly maintain and use trial-specific spatial information within a testing session). dHC-mPFC interactions have been shown to correlate with spatial working memory-guided task performance in rodents; however, there are no direct anatomical connections between the dHC and mPFC. The reuniens and rhomboid (RE/Rh) nuclei of the ventral midline thalamus are bi-directionally connected with the infralimbic, prelimbic, and anterior cingulate sub-regions of the mPFC, as well as the CA1 subfield of dHC. The efferent and afferent connections of the RE/Rh suggest that these thalamic nuclei may support working memory by modulating interactions between the dHC and mPFC. If RE/Rh support working memory by gating the flow of information between dHC and the mPFC, then functionally inactivating the RE/Rh should cause reduced synchrony in the dHC-mPFC network and concomitant performance impairments in spatial working memory tasks. We directly tested this prediction by simultaneously recording single units and local field potentials (LFPs) from CA1 of the dHC and the mPFC while rats performed a working memory-dependent delayed spatial alternation (DA) task in a T-maze. Prior to the recording session, RE/Rh were functionally inactivated by an intracranial infusion of the GABAA receptor agonist muscimol. Our results show that RE/Rh inactivation caused severe performance impairments that were accompanied by decreases in theta (4 - 12 Hz) power in the dHC LFP specifically during the time that the rat occupied the T-intersection of the maze prior to making a working memory-guided choice. dHC-mPFC LFP coherence, phase-amplitude coupling, and mPFC single-unit entrainment to hippocampal theta were also analyzed following RE/Rh inactivation. These

results provide a novel characterization of the mechanisms underlying memory-guided decision making by directly examining the relationship between thalamic gating of cortico-limbic interactions and spatial working memory performance.

Disclosures: H.L. Hallock: None. **A.L. Griffin:** None.

Nanosymposium

767. Synaptic Plasticity: Molecular and Circuit Mechanisms

Location: 147A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 767.08

Topic: F.02. Animal Cognition and Behavior

Support: CIHR

LDI/TD Bank Studentship to SEJH

Title: TrkA as a pharmacological target to modulate memory formation

Authors: *S. E. JOSEPHY HERNÁNDEZ[1], T. ABOULKASSIM[3], M. H. MAIRA ARCE[3], I. PIRVULESCU[2], H. U. SARAGOVI[2,3];
[2]Dept. of Pharmacol., [1]McGill Univ., Montreal, QC, Canada; [3]Lady Davis Inst. - Jewish Gen. Hosp., Montreal, QC, Canada

Abstract: Our research focuses on the Nerve Growth Factor (NGF) receptor tropomyosin-receptor-kinase A (TrkA). NGF and TrkA are expressed in the cholinergic neurons of the CNS and are key molecules for the maintenance of neuronal homeostasis, proliferation, circuitry formation, and memory. Accordingly, there is a linear correlation between decreased TrkA density and function, and disease progression in Alzheimer's disease (AD), Down's syndrome and cognitive impairment linked to ageing. Also, NGF transport has been shown to be impaired in AD. By synthetizing agonists of TrkA, our lab aims to stop and possibly reverse the memory deficits observed in these pathologies through neuronal rescue. Our lab reported a small molecule TrkA partial agonist termed D3, which activates TrkA and potentiates the effect of NGF. D3 improved long-term memory (LTM) in aged, memory-impaired rats; as well as learning and short-term memory (STM) in an APP-overexpressing mouse model of memory-impairment. However, paradoxically, D3 impaired LTM in healthy, young, wild type mice without effects on learning or STM, and without signs of toxicity. Here we report putative underlying pathways leading to this impairment. Young wild type mice were treated with

intraventricular D3 for 2 weeks and their brains were studied biochemically and by immunofluorescence. The mice had altered signaling pathways in the hippocampus, a memory relay organ, but not in the cortex. We focused on molecules that are downstream of TrkA and play an important role in memory formation. We detected significantly increased pAkt, CREB and Erk5 in the hippocampus. Also, we noted a decrease in BrdU+ neurons in the CA1 region suggesting a defect in migration or survival of neurons that replicated during treatment. We hypothesize that this disruption of the normal (wild type) TrkA/NGF homeostasis may cause the LTM impairment in wild type mice. This work will shed light on the mechanisms elicited by D3 and its possible use (or that of similar molecules) as a treatment for memory-related pathologies.

Disclosures: S.E. Josephy Hernández: None. **T. Aboulkassim:** None. **M.H. Maira Arce:** None. **I. Pirvulescu:** None. **H.U. Saragovi:** None.

Nanosymposium

767. Synaptic Plasticity: Molecular and Circuit Mechanisms

Location: 147A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 767.09

Topic: F.02. Animal Cognition and Behavior

Support: Gladstone Institutes

Hillblom Graduate Fellowship

Title: Changes in key memory-associated hippocampal oscillations in human apoE4 knock-in mice

Authors: *A. GILLESPIE[1], L. TONG[1], M. KARLSSON[1], K. KAY[2], Y.-H. LIN[1], S. YOON[1], L. FRANK[2], Y. HUANG[1];
[1]Gladstone Inst. of Neurolog. Disease, UCSF, San Francisco, CA; [2]Univ. of California, San Francisco, San Francisco, CA

Abstract: The apolipoprotein(apo)E4 variant of the APOE gene is the major genetic risk factor for Alzheimer's disease (AD): humans carrying the apoE4 allele have a substantially higher risk of developing cognitive decline during aging compared to others with the neutral apoE3 allele. Likewise, aged mice carrying the human apoE4 gene in place of the endogenous mouse apoE gene show learning and memory deficits compared to knock-in (KI) mice expressing human apoE3. Furthermore, apoE4-KI mice show an exacerbated age-related loss of GABAergic

interneurons specifically in the hilus of the hippocampus. While the severity of interneuron loss correlates with the extent of learning and memory impairment, the network mechanisms linking the cellular and behavioral manifestations of apoE4 expression are not understood. To investigate the functional impact of hilar interneuron loss on hippocampal network activity, we used chronic 32-channel silicon probes, which allow simultaneous local field potential (LFP) recordings across subregions within the dorsal hippocampus during behavior. Sharp wave ripple (SWR) events have been shown to play a crucial role in memory consolidation and reactivation, and we have found evidence for a link between apoE4 expression, SWR alterations, and behavioral impairment. At 12 months of age, apoE4-KI mice show a decrease in the rate of SWRs during putative sleep in a familiar environment compared to apoE3-KI mice. This decrease is even more pronounced during awake stillness in a novel environment. This effect is exacerbated with age, as 18-month-old apoE4-KI mice show an even further reduction of SWR rate during both sleep and awake stillness. These differences in SWR rate are predictive of multiple measures of learning and memory performance in the Morris water maze both within and across genotype groups, suggesting that the reduction of SWRs may have behavioral consequences. Additionally, we investigated whether the remaining SWRs in the apoE4-KI mice were similar to those of apoE3-KI mice. Increases in low gamma (30-50Hz) power are seen during SWRs, and low gamma coherence across subregions is a signature of high fidelity memory replay events. Low gamma power was dramatically reduced during SWRs in the CA1 stratum radiatum of apoE4-KI mice compared to apoE3-KI mice, suggesting that the remaining SWRs in these mice may not represent high quality replay events. Our results suggest that interneuron loss in apoE4-KI mice drives alterations in SWRs and associated memory replay, and that these alterations contribute to the observed learning and memory deficits and potentially to AD pathogenesis.

Disclosures: **A. Gillespie:** None. **L. Tong:** None. **M. Karlsson:** None. **Y. Lin:** None. **S. Yoon:** None. **K. Kay:** None. **L. Frank:** None. **Y. Huang:** None.

Nanosymposium

767. Synaptic Plasticity: Molecular and Circuit Mechanisms

Location: 147A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 767.10

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R21MH101579

NIH Grant R01MH073991

Title: Nicotine improves probabilistic learning via enhancing reward-associative learning , an effect attenuated in alpha 7 nAChR null mutants: A mechanism for alpha 7 nAChR agonist effects on negative symptoms in schizophrenia?

Authors: *J. W. YOUNG[1,2], K. HIGA[1], A. GRIM[1], M. A. GEYER[1,2];
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Abstract: To date, no treatments have been developed for negative symptoms in patients with schizophrenia despite these symptoms being linked to everyday functioning. This failing could stem from limited laboratory-based measurements that quantify negative symptoms. Recently, it was discovered that aspects of negative symptoms relate to poor reward-associative learning (RAL) as measured by probabilistic learning. Identifying treatments that enhance RAL may therefore treat negative symptoms. Patients with schizophrenia preferentially smoke nicotine at high rates, suggesting they may experience benefits from this 'self-medication'. We examined whether nicotine could improve RAL in mice. Because nicotine is the prototypical ligand for the nicotinic acetylcholine receptors (nAChR), we also determined whether nicotine could improve RAL in alpha7 nAChR mutant mice. Male alpha7 nAChR wildtype (WT; n=30), heterozygous (HT; n=27), and knockout (KO; n=27) mice were trained to respond for reward in either of two locations. Once responding reliably, these mice were baseline-matched by genotype into 3 groups, treated (s.c.) with vehicle or nicotine (0.03 or 0.3 mg/kg) 10 min prior to testing in a probabilistic learning task. In this task responses to the target stimulus were rewarded or punished at a 80/20 probability, and vice-versa for responses to the non-target stimulus. The primary outcome measure was trials to criterion (9 target responses in a row) and target win-stays reflecting RAL. Performance was analyzed using a two-way ANOVA with nicotine and genotype as between-subjects factors. Nicotine significantly improved trials to criterion ($F(2,75)=4.9$, $p<0.05$). No main genotype effect was observed ($F<1$, ns). In planned comparisons, nicotine improved trials to criterion in WT ($F(2,27)=3.8$, $p<0.05$), HT ($F(2,24)=3.5$, $p<0.05$), but not KO ($F<1$, ns) mice. Similarly, nicotine improved RAL ($F(2,75)=6.5$, $p<0.005$) in WT ($F(2,27)=4.2$, $p<0.05$), tended to in HT ($F(2,24)=3.2$, $p=0.061$), but did not in KO mice ($F<1$, ns). No effects of nicotine were observed on punishment-associative learning. Acute nicotine (0.3 mg/kg) improved learning in mice, an effect that was attenuated in alpha7 nAChR KO mice. Importantly, this improvement was driven by enhancing reward-associative learning in WT and HT, but not KO mice. These data support a putative role for alpha7 nAChR in reward-associative learning. Furthermore, these studies support recent clinical trials demonstrating that alpha7 nAChR agonists could treat negative symptoms in schizophrenia. Future studies will examine whether alpha7 nAChR agonists will similarly improve learning, and delineate their mechanism of action.

Disclosures: **J.W. Young:** A. Employment/Salary (full or part-time):; University of California, San Diego. 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, Omeros. F. Consulting Fees (e.g., advisory boards); Amgen. **K. Higa:** None. **A. Grim:** None. **M.A. Geyer:** A. Employment/Salary (full or part-time):; University of California, San Diego. 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.; Intracellular Therapeutics, Johnson & Johnson. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); San Diego Instruments. F. Consulting Fees (e.g., advisory boards); Abbott, Cerca, Dart Neuroscience, Merck, Omeros, Takeda, Teva.

Nanosymposium

767. Synaptic Plasticity: Molecular and Circuit Mechanisms

Location: 147A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 767.11

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant GM088801

NIH Grant AG041274

NIH Grant AG038994

Alzheimer's Association

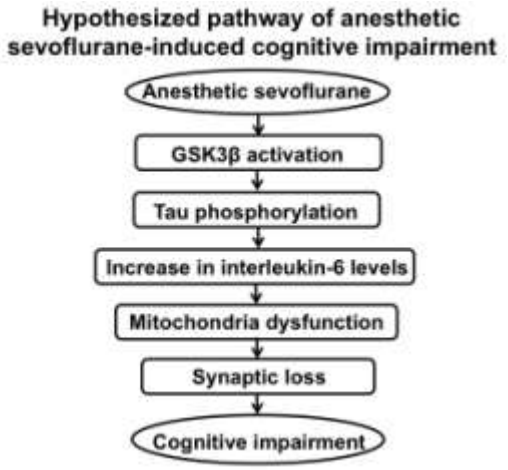
Cure Alzheimer's Fund

Title: Anesthetic sevoflurane induces cognitive impairment in young mice through Tau phosphorylation

Authors: J. ZHANG[1], Y. DONG[1], G. CROSBY[2], D. CULLEY[2], Y. ZHANG[1], *Z. XIE[1]; [1]Anesthesia, Critical Care and Pain Med., Massachusetts Gen. Hosp. and Harvard Med. Sch., Charlestown, MA; [2]Brigham & Women's Hosp., Boston, MA

Abstract: Children who have exposures to anesthesia have a higher risk of developing learning disability (Sun et al., 2010). However, the causes and underlying mechanisms remain to be

determined. Tau protein phosphorylation plays an important role in Alzheimer's disease dementia and cognitive dysfunction (Sheng, Sabatini and Sudhof, 2013). We therefore set out to determine the effects of sevoflurane on Tau phosphorylation, its up-stream mechanisms and down-stream consequences. Six day-old wild-type (WT), Tau knockout (KO), interleukin-6 KO, and cyclophilin KO mice were treated with 3% sevoflurane two hours daily for three days. The effects of the sevoflurane anesthesia were determined by Western blot analysis, ELISA, electron microscopy, and Morris Water Maze. The sevoflurane anesthesia induced Tau phosphorylation, enhanced activation of glycogen synthase kinase 3β (GSK3β) (the kinase related to Tau phosphorylation), caused mitochondrial dysfunction (ROS elevation, reduction in mitochondrial membrane potential and ATP levels), and decreased levels of postsynaptic density protein 95 (PSD-95) and synapse number in the hippocampus, as well as induced cognitive impairment in the mice. GSK3β inhibitor lithium mitigated the sevoflurane-induced adverse effects. The sevoflurane anesthesia did not induce an elevation of interleukin-6 levels, mitochondrial dysfunction, reduction in PSD-5 levels and synapse number in the hippocampus, or cognitive impairment in the Tau KO mice. The sevoflurane anesthesia only induced GSK3β activation and Tau phosphorylation in the interleukin-6 KO mice. In the cyclophilin KO mice, the sevoflurane anesthesia caused GSK3β activation, Tau phosphorylation, and interleukin-6 elevation, but no other effects. These data suggest that sevoflurane activates GSK3β in the hippocampus, which induces Tau phosphorylation, leading to interleukin-6 elevation. The elevated interleukin-6 then prompts mitochondrial dysfunction and synaptic loss, causing cognitive impairment in the mice as demonstrated in the Figure.



Disclosures: J. Zhang: None. **Y. Dong:** None. **G. Crosby:** None. **Y. Zhang:** None. **Z. Xie:** None. **D. Culley:** None.

Nanosymposium

767. Synaptic Plasticity: Molecular and Circuit Mechanisms

Location: 147A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 767.12

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH-R01NS060123

Title: Role of NRBF2 in modulating Class III-PI3K/autophagy activity and memory in mice

Authors: *J. LU, Z. YUE;
Dept. of Neurol. & Neurosci., Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: Background: Autophagy is a conserved lysosomal degradation pathway for recycle of intracellular content and energy. Autophagy is required for neuronal survival, and its dysfunction is implicated in neurodegeneration. PI3K-III complex is essential in controlling autophagy and endocytosis. In the previous study, we have identified a new component of PI3K-III complex: Nuclear receptor-binding factor 2 (NRBF2). Method: To understand the physiological role of NRBF2 in autophagy regulation and central nervous system, we generated NRBF2 KO mice. Through a combination of biochemical, cell biological and behavioral experiments, we dissected the role of NRBF2 in regulating PI3K-III complex and autophagy activity, and examined the effect of loss of NRBF2 on memory in mutant mice. Results: In the NRBF2 KO mice brain, both total and Atg14L-linked VPS34 kinase activity is dramatically impaired, concomitant with disassembly of PI3K-III complex. NRBF2 KO mice survive normally but display defects in spatial and fear memory. p62/SQSTM1, the substrate of autophagy, is accumulated in the brain and forms clustered granular extracellular structure specifically in the hippocampus of NRBF2 KO mice. Conclusions: Our study identified NRBF2 as a novel physiological autophagy regulator through modulating PI3K-III complex assembly and activity. NRBF2 and PI3K-III may participate in the cellular pathways in hippocampal neurons that control memory. (This study is supported by NIH-R01NS060123 awarded to Zhenyu Yue)

Disclosures: J. Lu: None. **Z. Yue:** None.

Nanosymposium

767. Synaptic Plasticity: Molecular and Circuit Mechanisms

Location: 147A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 767.13

Topic: B.08. Synaptic Plasticity

Support: NIH Grant R01 MH082876

Title: Circadian gene disruptions in midbrain regions alter excitatory synaptic plasticity and mood-related behavior in rodents

Authors: *P. K. PAREKH[1], A. OZBURN[1], M. SIDOR[2], S. SPENCER[3], R. LOGAN[1], J. KERN[1], Z. LIU[1], Y. HUANG[1], C. MCCLUNG[1];
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Abstract: The circadian molecular clock regulates monoaminergic systems that control mood and motivated behaviors. In this study, we investigated how disruptions in the core circadian genes, *Clock* and *Npas2*, lead to physiological alterations in mesolimbic circuitry contributing to manic-like and anti-anxiety behavior. Using *Clock* mutant mice (*Clock*19), a model of bipolar mania, we measured excitatory synaptic strength of medium spiny neurons (MSNs) in the nucleus accumbens (NAc), an area that receives a dense dopaminergic projection from the ventral tegmental area (VTA). Our previous studies indicate that *Clock* disruption in the VTA leads to abnormal dopaminergic signaling which may alter plasticity in downstream regions including the NAc. Additionally, we examined the effect of viral-mediated knock down (KD) of the homologous gene, *Npas2,* in the NAc in C57BL/6J mice on anxiety-related behavior and synaptic strength. *Clock*19 mice showed increased daytime levels of TH mRNA and protein in the VTA as well as increased extracellular dopamine in the NAc during the light cycle. They also displayed reduced AMPA-mediated synaptic strength of MSNs compared with WT animals. *Npas2* KD in the NAc resulted in an altered locomotor response to novelty and reduced anxiety-like behavior in the Light/Dark Box. Additionally, KD of *Npas2* produced an increase in mEPSC amplitude in NAc MSNs compared with scramble virus. The results of our study indicate an important role for circadian transcriptional mechanisms in the regulation of synaptic transmission in mesolimbic brain regions and the control of mood and reward related behavior in rodents.

Disclosures: P.K. Parekh: None. **A. Ozburn:** None. **M. Sidor:** None. **S. Spencer:** None. **R. Logan:** None. **J. Kern:** None. **Z. Liu:** None. **Y. Huang:** None. **C. McClung:** None.

Nanosymposium

768. APP Functions and Processing Pathways

Location: 150B

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 768.01

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

Support: T32AG000269

IIRG-12-241179

Title: Activation of the nuclear receptor PPARα exacerbates ADAM10-mediated proteolysis of APP

Authors: *G. T. CORBETT;
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Abstract: Genetic, molecular, pathological and biomarker studies suggest the amyloid precursor protein (APP) derivative β-amyloid (Aβ) plays an important role in the pathogenesis Alzheimer's disease (AD) onset. Generation of 40-42 amino acid Aβ is contingent upon sequential proteolytic processing of APP by β-secretase-1 (BACE1) and γ-secretase. Endosomal proteolysis of APP by BACE1 generates C-terminal (C99 or C89) fragments which are subsequently cleaved by γ-secretase to release Aβ peptides. Conversely, APP can also be cleaved by α-secretase, a disintegrin and metalloproteinase 10 (ADAM10), precluding BACE1 processing and generation of Aβ. Proteolytic activity of ADAM10, which occurs between the K16 and L17 extracellular residues of the APP sequence, generates a C-terminal (C83) fragment and a secreted form of APP (sAPPα). The C83 fragment is further cleaved by γ-secretase resulting in a non-neurotoxic p3 fragment. Low blood platelet ADAM10, low CSF sAPPα and improper ADAM10 trafficking to the membrane have been observed in AD patients versus healthy controls and missense mutations associated with ADAM10 have been proposed as indicators of late onset AD. Previous studies in animal models of AD suggest that genetic overexpression of ADAM10 decreases Aβ load, augments sAPPα release and improves Aβ-mediated impairments in long term potentiation. In light of these findings, induction of ADAM10 represents a plausible means to combat the earliest stages of Aβ production. However, the mechanisms by which ADAM10 is regulated remain poorly understood. Genetic analysis of ADAM10 promoter variants identified a PPAR response element (PPRE) consensus sequence proximal to the ADAM10 transcription start site, suggesting PPARα as a possible transcriptional regulator of ADAM10. Initially identified for its role in the breakdown of fatty acids and cholesterol in the liver, PPARα is capable of regulating the transcription of many genes in a tissue- and ligand-specific manner. Accordingly, transgenic mice null for PPARα (PPARα-/-), but not PPARβ (PPARβ-/-), are deficient for membrane-bound ADAM10, but not ADAM17 or BACE1, in the cortex and hippocampus relative to wild-type controls. Agonists of PPARα, but not PPARβ or PPARγ, dose-dependently elevate ADAM10 mRNA and protein expression and ADAM10-mediated release of sAPPα in wild-type neurons. Furthermore, young 5XFAD mice crossed with PPARα-/- mice (5X/α-/-) display exacerbated cortical and hippocampal Aβ load relative to age-matched 5XFAD mice. Our findings suggest

the lipid-lowering protein PPARα may be a critical factor in regulating neuronal ADAM10 expression and thus, nonamyloidogenic proteolysis of APP.

Disclosures: G.T. Corbett: None.

Nanosymposium

768. APP Functions and Processing Pathways

Location: 150B

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 768.02

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

Support: CIHR MOP-82890

Title: Arf6 controls the transport of APP to lysosomes by macropinocytsis, where it is cleaved into beta amyloid

Authors: *S. H. PASTERNAK[1,2,3], C. SEAH[4], W. TANG[2,4], J. H. K. TAM[2,4], S. P. CREGAN[2,4], S. P. MEAKIN[4,5];
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Abstract: Alzheimer's disease (AD) is characterized by the deposition of Beta-Amyloid (Aβ) peptides in the brain. Aβ is produced by cleavage of the Amyloid Precursor Protein (APP) by β- and γ- secretase enzymes after APP is internalized from the cell surface. The rapid internalization of APP and subsequent appearance of Aβ suggest that endosomes are an important compartment for this processing. We have found that APP and γ-secretase proteins and activity are highly enriched in the lysosome. We have also found that in addition to its rapid transport to endosomes, cell surface APP can be rapidly internalized and transported to the lysosome by a process that bypasses early and late endosomes. Here we show using confocal microscopy and live cell imaging that APP can be internalized via large (>500nm) vesicles that are too large to be classical transport vesicles. These vesicles then fuse with LAMP1 or LAMP2-labeled lysosomes. When viewed by electron microscopy, this process is reminiscent of macropinocytosis. APP internalization is blocked by an siRNA against the regulatory protein Arf6. Overexpression of Arf6 containing a dominant negative mutation blocks also lysosomal transport without affecting classical internalization to rab5-labled early endosomes. Finally, we show that blocking this lysosomal transport pathway significantly reduces Aβ production. This work suggests that the lysosome may be an important source of Aβ production.

Disclosures: **S.H. Pasternak:** None. **C. Seah:** None. **W. Tang:** None. **J.H.K. Tam:** None. **S.P. Cregan:** None. **S.P. Meakin:** None.

Nanosymposium

768. APP Functions and Processing Pathways

Location: 150B

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 768.03

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

Support: NS062184

MH096093

Beckman Institute

Title: Axonal transport is altered in aging mice with and without plaques induced by overexpression of human APP

Authors: E. L. BEARER[1,2], A. GONZALES[1], F. CHAVES[1], *R. E. JACOBS[3];
[1]The Univ. of New Mexico, Albuquerque, NM; [2]Caltech, Pasadena, CA; [3]Beckman Inst., Caltech, PASADENA, CA

Abstract: Axonal transport is critical for proper neuronal function. Components of the cellular transport machinery, amyloid precursor protein (APP) and tau, are also involved in the pathogenesis of Alzheimer's disease (AD), suggesting that defects in transport may contribute to cognitive impairment and neurodegeneration. We pioneered manganese-enhanced MRI (MEMRI) track tracing to measure axonal transport dynamics in the optic nerve and in the central nervous system (Bearer et al. 2007a and b, Bearer 2009). We now investigate the dynamics of transport in the hippocampus to forebrain circuit of aging mice with and without Abeta plaques resulting from 3-fold over-expression of $APP^{Swe/Ind}$. Aged mice (13-15 mo, n=16) were injected with 5 nL of aqueous $MnCl_2$ in CA3 of the right hippocampus and imaged in a Bruker 11.7T MR scanner with a T_1-weighted pulse sequence at increasing time points post injection (1h, 7h, 25h). After imaging, mice were sacrificed either for histology or biochemistry. A one-way within subject ANOVA for each genotype produced a statistical parametric map that revealed robust differences in transport between young wildtype, old wildtype, and old $APP^{Swe/Ind}$ expressing mice. Accumulation of Mn^{2+} at distant sites, medial septal nucleus (MSN) and contralateral hippocampus (CH), was significantly decreased with aging. Surprisingly the

volume of Mn^{2+} enhancement in old mice overexpressing mutant APP was increased compared to age-matched non-expressors, despite the presence of plaques. A region of interest (ROI) analysis to detect the degree of intensity difference in the MSN of the old WT and over-expressors revealed an $APP^{Swe/Ind}$ expression-by-time interaction ($p<0.001$) with decreased transport at 7h in the old WT, consistent with the SPM. As confirmed by microscopy, the cortices of overexpressing mice were dense with plaques. In contrast, the density of cholinergic neurons in the MSN was increased in old over-expressors compared to old WT. We conclude that APP overexpression enhances transport even in the presence of plaque. Supported by NS062184 and MH096093.

Disclosures: E.L. Bearer: None. **R.E. Jacobs:** None. **F. Chaves:** None. **A. Gonzales:** None.

Nanosymposium

768. APP Functions and Processing Pathways

Location: 150B

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 768.04

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

Support: DFG, FOR1332

Title: Amyloid Precursor Protein dimerization and synaptogenic function depend on copper binding to the growth factor like domain

Authors: *S. KINS[1], F. BAUMKÖTTER[1], N. SCHMIDT[1], S. SCHILLING[1], R. WEBER[1], C. VARGAS[1], S. KELLER[1], S. EGGERT[1], K. WILD[2];
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Abstract: Accumulating evidence suggests that the amyloid precursor protein (APP) has an essential synaptic function. APP synaptogenic function depends on trans-directed dimerization of the extracellular E1 domain encompassing a growth factor-like domain (GFLD) and a copper-binding domain (CuBD). Here we report the 1.75 Å crystal structure of the GFLD in complex with a copper ion bound with high affinity (KD = 28 nM) to an extended hairpin-loop at the dimerization interface. In co-immunoprecipitation assays increased intracellular copper levels promotes APP interaction, whereas mutations in the copper binding sites of either the GFLD or CuBD result in a drastic reduction in APP cis-orientated dimerization. Further, we provide evidence that copper is essential and sufficient to induce reversible trans-directed dimerization of

highly purified APP extracellular domain coupled to agarose beads. Finally we show that expression of APP in HEK293 cells promotes presynaptic differentiation of contacting axons of primary neurons and that this synatogenic activity depends on dimerization and copper binding. Together, these findings demonstrate that copper binding to the GFLD of APP is required for APP cis- / trans-directed dimerization and APP synaptogenic function in a mixed co-culture assay. Thus neuronal activity or disease-associated changes in copper homeostasis likely go along with altered synaptic function of APP.

Disclosures: **S. Kins:** None. **F. Baumkötter:** None. **N. Schmidt:** None. **S. Eggert:** None. **S. Schilling:** None. **R. Weber:** None. **S. Keller:** None. **C. Vargas:** None. **K. Wild:** None.

Nanosymposium

768. APP Functions and Processing Pathways

Location: 150B

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 768.05

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

Support: NIH/NIA Grant AG034980

Title: Amyloid precursor protein (APP) activates Cullin-5 (Cul5) ubiquitin ligase

Authors: ***Y. CHEN**[1], H. C. LIU[2], J. CHEN[2];
[1]Geriatrics, Univ. Arkansas Med. Sci., LITTLE ROCK, AR; [2]Univ. of Arkansas for Med. Sci., Little Rock, AR

Abstract: The amyloid precursor protein (APP) is mutated in Alzheimer's disease (AD), but the molecular function of APP remains undefined. APP interacts with the nedd8-activating enzyme (NAE, originally known as APP-BP1). Here we demonstrate that APP regulates Cullin-5 (Cul5) ubiquitin ligase, which plays essential roles in terminating cytokine signaling. We demonstrate that APP deficiency causes the accumulation of inactive Cul5 in neurons, similar to the effect of inactivating NAE in ts41 cells. APP deficiency also impairs Cul5 ubiquitin ligase activity in downregulating JAK3 in brain tissues and in synaptic terminals. Furthermore, APP overexpression modestly increases Cul5 turnover whereas APP intracellular domain (AICD) has a dominant negative effect analogous to that of APP deletion or NAE inactivation. We show that primary neurons exposed to IL-1β upregulate the levels of Cul5 ligase substrate receptors, SOCS1 and SOCS3, which can be blocked by an IL-1R1 antagonist. These data suggest that APP is necessary for neurons to assemble functional Cul5 ubiquitin ligases upon exposure to

pro-inflammatory cytokines. We also present evidence that familial AD APP mutations may impair Cul5 stability and function. APP Swedish mutant APP_{695}(K595N/M596L) significantly decreases Cul5 stability and diminishes Cul5 ligase activity. Together, our data suggest that APP regulates Cul5 ubiquitin ligase activity upstream of NAE in a manner sensitive to APP dosage and mutations. The identification of this APP pathway provides insights into mechanism-based drug targets and biomarker discoveries for AD and related diseases.

Disclosures: Y. Chen: None. **H.C. Liu:** None. **J. Chen:** None.

Nanosymposium

768. APP Functions and Processing Pathways

Location: 150B

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 768.06

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

Title: Local application of β-amyloid(1-42) elicits hippocampal sub-region specific glutamate release in C57BL/6 mice

Authors: *K. N. HASCUP, E. R. HASCUP;
Southern Illinois Univ. Sch. of Med., Springfield, IL

Abstract: Alzheimer's disease (AD) is an age-related neurodegenerative disorder characterized by hippocampal atrophy that leads to progressive cognitive and functional decline and eventual death. A major hallmark of AD is the presence of senile plaques, of which, high molecular weight oligomers of B-amyloid (Aβ) is the major constituent. Aβ is a 39-42 amino acid peptide whereby the longer 42 amino acid isoform is known to aggregate more readily and considered the most neurotoxic. However, recent evidence suggests that soluble $A\beta_{1-42}$ may play a key role in modulating neurotransmitter release. Specifically, studies have shown that $A\beta_{1-42}$ binds nicotinic acetylcholine receptors (nAChRs) located presynaptically on glutamatergic terminals and may modulate glutamate release. For this reason, we examined the effects of the human isoform of $A\beta_{1-42}$ on glutamate release in the CA1, CA3, and dentate gyrus (DG) of isoflurane anesthetized, 6-9 month old male C57BL/6 mice. We utilized an enzyme-based microelectrode array (MEA) selective for measures of L-glutamate with fast temporal (4 Hz) and high spatial (50 x 100 microns) resolution. To examine glutamate neurotransmission, $A\beta_{1-42}$ (0.1, 1.0, or 10.0 μM) was dissolved in physiological saline (pH ~7.4) and loaded into a micropipette positioned 100 microns away from pairs of recording sites on the MEA. Local application of 0.1 and 1.0 μM $A\beta_{1-42}$ (150 nl ± 50 nl; 1-2 seconds) elicited robust, reproducible glutamate signals in all

hippocampal sub-regions studied. Application of 0.1 μM $A\beta_{1-42}$ (n=8) significantly ($p<0.05$) increased the average maximal amplitude of glutamate release compared to saline (n=9) in the CA1 (3.3 ± 0.7 μM and 0.5 ± 0.1 μM; respectively) and DG (3.8 ± 1.1 μM and 0.5 ± 0.1 μM; respectively), but not in the CA3. A similar trend ($p=0.05$) was observed with 1.0 μM $A\beta_{1-42}$ (n=8) compared to saline (n=9) control in the CA1 (2.8 ± 1.0 μM and 0.5 ± 0.1 μM; respectively). 10.0 μM $A\beta_{1-42}$ did not show a significant change in glutamate levels in any of the brain regions examined. This is the first study to show that $A\beta_{1-42}$ elicits glutamate release and could help to explain the neurotoxicity observed in AD. Furthermore, the sub-regional differences observed may be an indication of varying susceptibility to the excitotoxic onslaught caused by $A\beta_{1-42}$. Future studies will examine the effects of inhibiting α7 and α4β2 nAChRs on $A\beta_{1-42}$ induced glutamate release.

Disclosures: K.N. Hascup: None. **E.R. Hascup:** None.

Nanosymposium

768. APP Functions and Processing Pathways

Location: 150B

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 768.07

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

Support: NIH NS078363

NIH AG025525

Title: Amyloid Precursor Proteins as regulators of G protein-dependent neuronal guidance

Authors: *P. F. COPENHAVER[1], J. A. ZWEIG[2], T. L. SWANSON[2], J. M. RAMAKER[2]; [2]Cell and Developmental Biol., [1]Oregon Hlth. & Sci. Univ., PORTLAND, OR

Abstract: The amyloid precursor protein (APP) is best known as the source of beta amyloid peptides that are associated with Alzheimer's disease (AD). In addition, APP has also been shown to regulate neuronal growth and motility in a variety of contexts, suggesting that the dysregulation of its normal functions might contribute to the pathology of AD. However, the mechanisms underlying APP-dependent responses in the nervous system are still controversial. In vitro studies have shown that APP can affect cellular behavior via activation of the heterotrimeric G protein Gαo, suggesting that APP might act as an unconventional G protein-coupled receptor, but compensatory interactions by other APP-related proteins have hindered an

analysis of this process in the mammalian brain. As an alternative strategy, we have used simpler model systems (Manduca and Drosophila) to demonstrate that the sole insect ortholog of APP (APPL) co-localizes with Gαo in the leading processes and synaptic terminals of developing neurons, and that the two proteins directly interact. In embryo culture, stimulation of both APPL and Gαo signaling functions to prevent neuronal growth into inappropriate regions of the nervous system, while Gαo activation regulates APP-Gαo interactions. Based on evidence that mammalian contactins can bind APP, we recently found that insect contactin is developmentally expressed within regions that are inhibitory to neuronal outgrowth, while treating migrating neurons with contactin-Fc fusion proteins inhibits their motile behavior. Likewise in cultured murine hippocampal neurons, stimulating APP signaling causes growth cone collapse in a Gαi/Gαo-dependent manner, indicating that this signaling pathway is evolutionarily conserved. Initial studies using human brain samples have also shown that that reduced APP-Gαo interactions correlate with the severity of Alzheimer's-related pathology, supporting the model that the loss of normal APP-dependent signaling may exacerbate neurodegenerative responses. We are now testing the hypothesis that contactins function as endogenous APP ligands that can activate APP-Gαo signaling in a context-dependent manner, providing a mechanism for regulating the motile behavior of neurons in both the developing and mature brain.

Disclosures: P.F. Copenhaver: None. **J.A. Zweig:** None. **T.L. Swanson:** None. **J.M. Ramaker:** None.

Nanosymposium

768. APP Functions and Processing Pathways

Location: 150B

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 768.08

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

Support: DFG KO 1898/6- 1 and 10/1

Title: Neuroprotection by secreted APP requires expression of membrane-tethered holo-APP

Authors: *D. KOGEL[1], N. ROHNER[1], A. KUNDU[1], S. KINS[2], U. MULLER[3], C. BEHL[4]; [1]Frankfurt Univ. Hosp., Frankfurt, Germany; [2]Div. of Human Biol. and Human Genetics, Univ. of Kaiserslautern, Kaiserslautern, Germany; [3]Inst. of Pharm. and Mol. Biotechnology, Univ. of Heidelberg, Heidelberg, Germany; [4]Inst. for Pathobiochemistry, Mainz Univ., Mainz, Germany

Abstract: Objectives: In addition to the detrimental effects of Abeta, loss of the physiological functions of the amyloid precursor protein (APP) might be crucially implicated in reduced neuronal plasticity, diminished synaptic signaling and enhanced susceptibility of neurons to cellular stress during brain aging. Here we investigated the neuroprotective function of the soluble APP ectodomain sAPPα, which is generated by cleavage of APP by α-secretase along the non-amyloidogenic pathway, and the possible roles of holo-APP and APLPs in this context. To this end, we analyzed the modulatory effects of sAPPα on stress and survival signaling and cell death in KD/KO models. Methods: Effects of sAPPα and its subdomain E1 (His-tagged polypeptides purified from Pichia pastoris) on stress/survival pathways and cell survival/cell death were investigated in human neuroblastoma SH-SY5Y cells (controls, APP/APLP1/APLP2 KD), primary hippocampal neurons and fibroblasts. Results: Here we demonstrate that sAPPα and E1 can activate survival signaling and inhibit the GSK-3β and JNK stress signaling pathways. Depletion of APP by stable lentiviral knockdown indicated that sAPPα and E1 depend on the expression of membrane-bound holo-APP to exert their neuroprotective functions under stress conditions. In contrast, APLP1 and APLP2 were not necessary for sAPPα/ E1-mediated activation of cell survival. Conclusions: Our data suggest a physiological role of sAPPα in controlling cell death activation in response to neurotoxic stress. This beneficial effect of sAPPα depends on holo-APP and is associated with the inhibition of pro-apoptotic stress kinases and activation of survival signaling.

Disclosures: D. Kogel: None. **N. Rohner:** None. **A. Kundu:** None. **S. Kins:** None. **U. Muller:** None. **C. Behl:** None.

Nanosymposium

768. APP Functions and Processing Pathways

Location: 150B

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 768.09

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

Support: NIH AG00538

NIH AG033069

Cure Alzheimer Fund

Title: A unique conformation of amyloid is associated with cerebrovascular amyloid deposits and smooth muscle cell degeneration

Authors: *R. ALBAY, III, J. CARRANZA, S. MILTON, C. G. GLABE;
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Abstract: The deposition of insoluble amyloid-beta (Aβ) aggregates as extracellular plaques, along with intracellular neurofibrillary tangles (NFT) are characteristic hallmarks of Alzheimer's disease (AD). Several types of cerebral amyloid angiopathy (CAA) have been identified and often have co-morbidity with neurodegenerative disease. Sporadic CAA caused by the accumulation of Aβ is the most common in elderly individuals as well as patients with AD. Amyloid aggregates are heterogenous in size, morphology, solubility and underlying beta sheet structure and the understanding of which aggregate conformations are pathologically relevant to AD and CAA would be a tremendous advantage in developing therapeutics to slow or eliminate the disease. Previously we reported that among a panel of conformation dependent antibodies, mOC31 exclusively recognized vascular amyloid and not parenchymal plaque deposits in elderly, AD and CAA human tissue sections. These findings demonstrated that a subset of vascular amyloid had a unique conformation that could be recognized by the mOC31 monoclonal antibody. Here, we report that the mOC31 immunoreactivity is associated with roughly 5% of all vessels and as high as 44% of smooth muscle containing vasculature. Furthermore, the arterioles and venules responsible for maintaining homeostatic blood flow suffer smooth muscle degeneration in the presence of mOC31 immunoreactive amyloid. Amyloid aggregates are conformationally diverse and the immunological response to these amyloids have been instrumental in advancing our understanding of the role conformational strains play in disease progression, exemplified by mOC31 immunoreactive vascular amyloid.

Disclosures: R. Albay: None. **J. Carranza:** None. **S. Milton:** None. **C.G. Glabe:** None.

Nanosymposium

768. APP Functions and Processing Pathways

Location: 150B

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 768.10

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

Support: NIH Grant AG039668 (Z.L.M.)

NSF Grant IOS-1347090 (V.M., Z.L.M.)

New Jersey Health Foundation Grants (Z.L.M., V.M.)

Title: Convergent pathogenic mechanisms of Alzheimer's disease and ALS: Peripherin-dependent transport of sAPP, likely with neurofilament-associated endoplasmic reticulum tubules

Authors: *V. MURESAN, Z. LADESCU MURESAN;
Pharmacol. and Physiol., New Jersey Med. School, Rutgers, The State Univ. of New Jersey, Newark, NJ

Abstract: Amyotrophic lateral sclerosis (ALS) is a debilitating neurodegenerative disorder of motor neurons, leading to deterioration of neuromuscular junctions, by poorly understood mechanisms. One pathological trait of ALS-afflicted neurons is the disorganization of neurofilaments (NFs), which, in some forms of ALS, is caused by mutations in the NF gene, *peripherin* (*NEF4; PRPH1*). Recently, we reported that the disruption of peripherin neurofilaments - as occurs in ALS - alters the metabolism of amyloid-β precursor protein (APP), a protein linked to the pathology of Alzheimer's disease (AD) (Muresan et al., 2014, Neurodegener. Dis., 13:122-125). Here, with brainstem-derived, mouse neuronal cells (CAD), we show that the transport of the N-terminal fragment of APP, sAPP, requires peripherin NFs. We show that sAPP is generated by cleavage of APP by β-secretase in the soma, in a juxtanuclear, endoplasmic reticulum (ER) compartment that colocalizes with, and is stabilized by, peripherin. Unlike the C-terminal fragments of APP, which are carried into neurites with typical transport vesicles, sAPP becomes localized to elongated structures that extend from the soma to the growth cone, and colocalize with peripherin NFs. These structures contain the tubular ER marker, Reticulon 4 (Rtn4) - an AD-relevant protein - but not the ER resident proteins, calreticulin and protein disulfide isomerase (PDI). This suggests that the sAPP, generated in the soma, is targeted to the neurites by a novel form of transport that does not relay on classical transport vesicles, but rather on a specialized, tubular ER compartment, which extends into processes along peripherin NFs. Knocking down peripherin with siRNA disrupts the NF network, and diminishes accumulation of sAPP and Rtn4 at neurite terminals, as does the selective disruption of acetylated microtubules. These results indicate that the tubular ER, loaded with sAPP, extends into neurites by associating with peripherin NFs, which themselves translocate along acetylated microtubules. This is the first report showing that an ER subcompartment could function in the long-distance transport of membrane and secretory proteins, and that peripherin NFs are implicated in such transport. Taken together, these results suggest that the neuronal degeneration in ALS could be the result of impeded ER-mediated transport, which relies on peripherin NFs - disrupted in ALS. We propose that a deficient ER translocation into neurites, preventing transport of disease-relevant proteins with essential function at the synapse, such as sAPP, or the RNA-binding proteins, TDP-43 and FUS, could be a mechanism of disease both in AD and ALS. .

Disclosures: **V. Muresan:** None. **Z. Ladescu Muresan:** None.

Nanosymposium

769. Huntington's Disease Mechanisms and Therapeutic Strategies

Location: 147B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 769.01

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Bristol Cardiff Neuroscience Collaboration grant

Title: Glucocorticoid signalling actions are disrupted in Huntington's disease

Authors: *C. T. UDEH[1], S. BROOKS[3], N. JANGHRA[3], S. DUNNETT[3], S. L. LIGHTMAN[2]; [1]HW LINE, [2]Henry Wellcome Lab. for Integrative Neurosci. and Endocrinol., Univ. of Bristol|910005591|0, Bristol, United Kingdom; [3]Brain Repair Group, Cardiff Univ., Cardiff, United Kingdom

Abstract: Huntington's disease (HD) is a progressive neurodegenerative condition characterised by a complex of symptoms that include behavioural (motor, cognitive and psychiatric), metabolic, and neuroendocrine dysfunctions. Over the last ten years, evidence has amassed validating malfunction of neuroendocrine axes (e.g. the HPA axis) as playing a fundamental role in disease progression. Thus, many patients present with disrupted pulsatile glucocorticoid (GC) secretion, triggered by HPA axis hyperactivity, as well as marked circadian disturbances. Furthermore, in-vitro research point towards dysfunctional glucocorticoid receptor (GR) signalling mechanisms in HD pathogenesis due to an exacerbation of disease characteristic pathologies, including protein aggregation and nuclear localization, mechanisms which can be modulated by the GR. This is important as aberrant GC actions for example dysregulated expression of key target genes, may contribute to some of the impairments evident in HD biochemistry. Although disease causing disruptions to functions of major GC target organs has been widely studied in animal models and even humans, the role of the GR in mediating some of the clear impediments seen in the disease has not been assessed in vivo. Here we present novel data showcasing abnormal diurnal responses in the hypothalamus, pituitary and liver of early stage Huntington disease transgenic mice of the the R6/1 strain, compared to wild-type animals. Firstly, we report a dysregulated diurnal rhythm of GC secretion in R6/1 animals. Secondly, we show for the first time that the transcriptional response to circadian rise in GCs is anomalous in these carriers of the mutant huntingtin gene, as evidenced by the disparate expression of well characterised glucocorticoid responsive genes, namely the clock gene period1 (per1) as well as serum and glucocorticoid-inducible kinase 1 (sgk1). Thirdly, we observed a marked dysregulation in the transgenic animals of phosphorylation of the GR at a specific residue on the receptor protein which serves as a mechanistic biomarker for GR activation. Taken together, our

data highlight the possibility that disrupted HPA axis activity in Huntington's disease may be an early characteristic of disease pathology and contribute to some of the maladaptive features of the condition. In light of these findings, targeted research into understanding the implications of the deviant GR signalling actions that might be present in HD may contribute towards the search for effective therapies in managing the condition.

Disclosures: C.T. Udeh: None. **S.L. Lightman:** None. **S. Dunnett:** None. **S. Brooks:** None. **N. Janghra:** None.

Nanosymposium

769. Huntington's Disease Mechanisms and Therapeutic Strategies

Location: 147B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 769.02

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Scripps Startup Funds

O'Keeffe Neuroscience Scholar Award

Title: Huntingtin regulates nutrient-mtorc1 signaling that exacerbates huntington's disease symptoms

Authors: *S. SUBRAMANIAM[1], M. BIAGIOLI[2], M. MACDONALD[2], S. SWARNKAR[1], N. SHAHANI[1], W. PRYOR[1];
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Abstract: Huntingtin (Htt) is a ubiquitously expressed protein, whose polyglutamine tract is encoded by the HTT CAG repeat expansion that causes Huntington disease (HD) with an early cell loss in the striatum, affecting motor and cognitive functions. Yet the physiological functions for Htt or its mechanisms for HD pathogenesis remain unclear. Here we demonstrate in striatal cells and mouse embryonic stem cell systems, that Htt activates mTORC1 (mechanistic-target of rapamycin kinase complex 1) signaling that is potentiated by N-terminal expanded polyglutamine fragment of Htt. While depletion of Htt attenuates amino acid-induced mTORC1 activity, the overexpression of Htt potentiates it. Pharmacological blockade of phosphatidylinositol-3-kinases or the depletion of Rheb GTPase using shRNA abolishes Htt fragment-mediated mTORC1 activity. In the presence of amino acids, Htt forms rapid perinuclear accumulations, binds to Rheb GTPase and enhances its interaction with mTOR.

Moreover, selective deletion of tuberous sclerosis 1 (TSC1), a negative regulator of mTORC1, in the striatum of a HD mouse model, TSC1flox/+/N171HD mice, causes increased severity of behavioral abnormalities and premature demise. Altogether, we demonstrate that Htt is a novel regulator of amino acid-induced mTORC1 activity that potentiates motor and cognitive defects in an HD mouse model (Pryor et al., revised).

Disclosures: S. Subramaniam: None. **M. Biagioli:** None. **M. Macdonald:** None. **S. Swarnkar:** None. **N. Shahani:** None. **W. Pryor:** None.

Nanosymposium

769. Huntington's Disease Mechanisms and Therapeutic Strategies

Location: 147B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 769.03

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: United States Public Health Service Grant MH18501

CHDI

Title: Loss of inositol polyphosphate multikinase contributes to neurodegeneration in Huntington's disease

Authors: *I. AHMED[1], J. I. SBODIO[1], M. M. HARRAZ[1], R. TYAGI[1], L. K. ALBACARYS[1], R. XU[1], J. GRIMA[1], S. KIM[2], B. D. PAUL[1], S. H. SNYDER[1];
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Abstract: Huntington's disease (HD) is a progressive neurodegenerative disorder caused by a poly-glutamine repeat expansion in the N-terminal region of the huntingtin gene. This mutation results in striatal atrophy from the selective loss of medium spiny neurons. Various processes may contribute to this selective neuronal dysfunction and death, including altered BDNF signaling. Prior work has indicated a role for inositol polyphosphate multikinase (IPMK) in the neuronal response to BDNF, but in the context of immediate early gene induction and neuronal differentiation. Thus, we sought to elucidate the role of IPMK in neurodegeneration. IPMK is a pleiotropic enzyme with inositol phosphate kinase and lipid kinase activities, which produce IP4 and IP5, as well as PIP3, respectively. IPMK also displays non-catalytic functions, including regulation of mTOR signaling and CBP/p300 mediated-transcription. We show that IPMK

expression is reduced in cellular and animal models of HD and, more importantly, in post-mortem HD striatal samples. This loss of IPMK is caused by alterations at both the transcriptional and protein levels. The striatal-enriched transcription factor Ctip2, which is depleted in HD, regulates IPMK expression. The stability of IPMK protein is also reduced in the cellular model of HD, possibly through its selective interaction with mutant huntingtin. Lastly, over-expression of IPMK rescues the metabolic activity deficit present in the cellular model of HD. This rescue is mediated by the lipid kinase activity of IPMK. Our findings imply that IPMK is an important player in the pathophysiology of HD. Furthermore, this signaling pathway provides a novel therapeutic target for HD and potentially other neurodegenerative diseases.

Disclosures: I. Ahmed: None. **J.I. Sbodio:** None. **M.M. Harraz:** None. **R. Tyagi:** None. **L.K. Albacarys:** None. **R. Xu:** None. **J. Grima:** None. **S. Kim:** None. **B.D. Paul:** None. **S.H. Snyder:** None.

Nanosymposium

769. Huntington's Disease Mechanisms and Therapeutic Strategies

Location: 147B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 769.04

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Evelyn Trust

Title: The microtubule associated protein Tau and its impact on cognition in Huntington’s disease

Authors: *R. VUONO, S. E. WINDER-RHODES, R. A. BARKER;
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Abstract: Huntington’s disease (HD) is a neurodegenerative disorder with an autosomal dominant pattern of inheritance. It is characterized by psychiatric disturbances, motor dysfunction and cognitive decline and the latter may even be present in the early stage of the disease, before the onset of overt motor features. The genetic mutation is characterized by an unstable expansion of a CAG repeat within the coding region of the IT-15 gene on chromosome 4p16.3. The gene encodes for a protein called huntingtin (HTT) and the mutation results in an elongated stretch of glutamine near the N-terminus of the protein. The consequence of carrying the HD mutation is a widespread neuronal dysfunction and neurodegeneration including the cerebral cortex and striatum. In HD patients, the number of CAG repeats range from 36-120 and

the longer the CAG repeat length the earlier the age of onset of the disease. In addition more than 24 other candidate genes have been identified which could modify HD onset and progression. One gene of interest that we have recently started to study is Tau, as this has been implicated in a range of other neurodegenerative disorders of the CNS. We have now found in a large cohort of HD patients that Tau haplotype has a significant effect on cognitive decline in HD. Furthermore using post mortem tissue we have also found altered tau isoforms expression as well as tau deposits in the cortex and striatum in HD brains compared to healthy-matched controls. All of this data suggests that there is maybe a link between mutant HTT and Tau in driving some aspects of the HD pathology. Acknowledgements: Evelyn Trust, European Huntington's Disease Network, NIHR Biomedical Research Centre Funding/Tissue Bank.

Disclosures: R. Vuono: None. **S.E. Winder-Rhodes:** None. **R.A. Barker:** None.

Nanosymposium

769. Huntington's Disease Mechanisms and Therapeutic Strategies

Location: 147B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 769.05

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: JPB Foundation Grant

Title: A link between polyglutamine protein load and cellular vulnerability in huntington's disease

Authors: *L. HACHIGIAN[1,3], A. HEILBUT[4,5], R. FENSTER[4,3], R. KULICKE[4,3], E. KOLACZYK[5,6], J. MESIROV[4,5], M. HEIMAN[3,4,2];
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Abstract: Huntington's disease (HD), the most common inherited neurodegenerative disorder, is caused by mutations in the *huntingtin* (*HTT*) gene, which encodes a poly-glutamine (polyQ) repeat protein. Despite widespread expression of the *HTT* gene, HD presents with massive neuronal cell loss in the striatum and deep layers of the cortex. This enhanced vulnerability of striatal and cortical neurons provides an opportunity to understand the molecular mechanisms contributing to pathophysiology in HD. Using the cell-type specific translating ribosome affinity purification (TRAP) methodology, we profiled dozens of neuronal cell types and determined

groups of genes that are differentially expressed in striatal and cortical neurons versus other, less-vulnerable cell types in HD. TRAP profiles of deep-layer cortical pyramidal neurons and striatal medium spiny neurons demonstrate a high level of expression of genes encoding non-HTT polyQ repeat proteins, as compared to neuronal cell types found in other, less vulnerable brain regions. As it has been previously demonstrated that polyQ peptides can influence HTT protein aggregation and cellular toxicity, and that polyQ proteins can be sequestered into mutant HTT aggregates, we reasoned that a higher level of expression of polyQ-encoding genes in striatal and cortical neurons may be linked to those cell types' enhanced cellular vulnerability in HD. In primary striatal neuronal cultures, overexpression of cortical- and striatal-enriched polyQ proteins led to an increase in mutant HTT aggregation. Analysis of HD human and mouse model tissue revealed that several cortical- and striatal-enriched polyQ-containing proteins co-aggregate with mutant HTT protein. Taken together, our data suggest that a high expression level of polyQ proteins (separate from HTT expression) characterizes cells that display enhanced vulnerability in HD, and that cortical- and striatal-enriched polyQ proteins can influence the kinetics of aggregation of and can also co-aggregate with mutant HTT protein. Finally, preliminary data suggest that loss of cortical- and striatal-enriched polyQ-protein function, due to co-aggregation with mutant HTT, contributes to the enhancement of the cellular vulnerability that is observed in HD in cortical and striatal regions.

Disclosures: L. Hachigian: None. **A. Heilbut:** None. **R. Fenster:** None. **R. Kulicke:** None. **E. Kolaczyk:** None. **J. Mesirov:** None. **M. Heiman:** None.

Nanosymposium

769. Huntington's Disease Mechanisms and Therapeutic Strategies

Location: 147B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 769.06

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CIHR grant MOP 84438

Title: Mutant Huntingtin resistant to cleavage at amino acid 586 preferentially binds p62 and is cleared more efficiently by autophagy

Authors: *D. E. EHRNHOEFER, S. LADHA, X. QIU, Y. T. N. NGUYEN, M. R. HAYDEN; Med. Genet., UBC, Vancouver, BC, Canada

Abstract: Huntington's Disease (HD) is a neurodegenerative disorder caused by the elongation of a CAG repeat in the HD gene encoding the huntingtin protein (Htt) over the threshold of 37 repeats. The expression of caspase cleavable mutant Huntingtin (mHtt) in vivo leads to the development of HD-like symptoms in transgenic YAC128 mice, however animals expressing mHtt resistant to caspase-6 (C6) cleavage at amino acid 586 (C6R mHtt) are fully rescued from all signs of pathology. Recent work has shown that the ablation of C6 activates autophagy in mouse models of HD, leading to more efficient degradation of mHtt in vivo. Since C6 activity is significantly reduced in the C6R mouse model of HD, we have investigated the possibility that improved clearance of the transgenic mHtt protein in C6R mice is responsible for their lack of pathology. In the liver, a tissue with high basal autophagy, protein levels of mHtt are the same in young C6R and YAC128 mice. However we find that mHtt levels increase in YAC128 but not C6R animals as the mice age, indicating that degradation mechanisms are progressively impaired in YAC128. Assessment of autophagy markers in the liver suggests that autophagy in young YAC128 mice is increased over baseline, which is not sustained with age and may lead to the observed buildup of mHtt protein. No increase in autophagy over wild-type levels was found in the livers of C6R animals, suggesting that the degradation of C6R mHtt does not require an increase in autophagic flux but rather baseline levels of autophagy are sufficient to clear C6R mHtt. p62 is an autophagy adaptor protein that links polyubiquitinated and aggregated substrates such as mHtt to LC3 and the autophagosome, thus mediating its degradation. The interaction between mHtt and p62 is therefore crucial for efficient autophagic degradation of mHtt, and we find that the interaction of p62 with C6R mHtt is twice as strong as its interaction with cleavable YAC128 mHtt. An increase in p62 binding was also observed for cleavable mHtt expressed in the presence of a caspase inhibitor, indicating that the lower binding efficiency for YAC128 mHtt is due to its cleavage by caspases. Our results show that cleavable mHtt in YAC128 mice requires upregulation of autophagy to prevent its accumulation, while C6R mHtt can be degraded more efficiently through its stronger interaction with p62. Preventing mHtt proteolysis at aa586 could therefore lead to a more efficient degradation of the disease protein and thus ameliorate disease phenotypes caused by the presence of mHtt protein in the CNS as well as in peripheral tissues.

Disclosures: **D.E. Ehrnhoefer:** None. **S. Ladha:** None. **X. Qiu:** None. **Y.T.N. Nguyen:** None. **M.R. Hayden:** A. Employment/Salary (full or part-time):; Teva Pharmaceuticals.

Nanosymposium

769. Huntington's Disease Mechanisms and Therapeutic Strategies

Location: 147B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 769.07

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH/NINDS Grant U24NS078370

Title: Proteomic analysis of HD iPS cells differentiated to striatal neurons

Authors: *S. AKIMOV[1], T. RATOVITSKI[1], C. TALBOT[2], R. N. COLE[3], V. B. MATTIS[4], C. SVENDSEN[4], L. M. THOMPSON[5], C. A. ROSS[1];
[1]Dept. of Psychiatry, Johns Hopkins University, Sch. of Med., Baltimore, MD; [2]Inst. for Basic Biomed. Sci., [3]Mass Spectrometry and Proteomics Facility, Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [4]Cedars-Sinai Regenerative Med. Inst., Los Angeles, CA; [5]Dept. of Psychiatry and Human Behavior, Univ. of California-Irvine, Irvine, CA

Abstract: Previously, we established and characterized an induced Pluripotent Stem (iPS) cell model for Huntington's disease (HD), a genetic neurodegenerative disorder caused by CAG repeat expansion in exon 1 of the Huntingtin (HTT) gene. Currently, we use iPS cell lines derived from the same and new donors using non-integrating method. We used iTRAQ (Isobaric tags for relative and absolute quantitation) proteome analysis to identify proteins with expression significantly changed in HD and control non-integrating iPS cells differentiated to medium spiny neurons. Our results demonstrate separation of HD versus control by Principal Component Analysis and Hierarchical clustering (heatmap). Using Ingenuity Pathway Analysis, we identified a number of pathways significantly changed in HD versus control cells. Particularly, glutamate receptors, calcium signaling pathways, and a number of pathways related to cellular metabolism were altered. The results are under validation by Western blotting and functional studies. We anticipate that these results will help to elucidate mechanisms of HD pathogenesis and identify new therapeutic targets in HD.

Disclosures: S. Akimov: None. **T. Ratovitski:** None. **C. Talbot:** None. **R.N. Cole:** None. **V.B. Mattis:** None. **C. Svendsen:** None. **L.M. Thompson:** None. **C.A. Ross:** None.

Nanosymposium

769. Huntington's Disease Mechanisms and Therapeutic Strategies

Location: 147B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 769.08

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: CHDI grant

Title: Autophagy related PI-3 Kinase III activity in Huntington's disease model and p62 phosphorylation in the degradation of PolyQ Htt mutant

Authors: *J. LIM[1], M. L. LACHENMAYER[2], Z. YUE[3];
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Abstract: Huntington's disease (HD) is a devastating neurodegenerative movement disorder, characterized as one of the polyglutamine (polyQ) disease family. Huntingtin protein (Htt) with polyQ expansion accelerates protein aggregation and emerging evidence shows that autophagy selectively degrades protein aggregates. Recent evidence shows the presence of selective autophagy, which plays an important role in the prevention of ubiquitinated protein overflow/aggregation and maintenance of homeostasis of cellular organelles. Although it has been shown that this form of autophagy is mediated through autophagy receptor proteins, (e.g. p62/SQSTM1, NBR1, ALFY), the signaling regulation of selective autophagy remains still elusive. Recently we have characterized the status of autophagy in mouse model brains of HD by analyzing two most important kinase systems for autophagy: lipid kinase beclin 1-Vps34 complex and protein ULK1 complex activities. In addition, we found that ULK1 directly regulates p62-mediated degradation of ubiquitinated proteins and polyQ-Htt mutant proteins by phosphorylating p62. We will report our findings and discuss how ULK1 signaling is linked to selective autophagy through p62 phosphorylation, a process relevant to the clearance of aggregate-prone disease proteins.

Disclosures: J. Lim: None. **M.L. Lachenmayer:** None. **Z. Yue:** None.

Nanosymposium

769. Huntington's Disease Mechanisms and Therapeutic Strategies

Location: 147B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 769.09

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH NRSA F32 NS081964

NIH R01 NS65874

Hereditary Disease Foundation

CHDI Foundation, Inc.

Title: Bexarotene activation of PPARδ function improves pre-clinical trial outcomes in a mouse model of Huntington's disease

Authors: *A. S. DICKEY[1], N. LOMAS[1], K. R. SAMPAT[1], D. N. SANCHEZ[1], E. MASLIAH[2], A. R. LA SPADA[1];
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Abstract: Huntington's disease (HD) is a relentlessly progressive autosomal dominant neurodegenerative disorder characterized by the development of involuntary movements, cognitive decline, and psychiatric illness. Recent studies by our laboratory and others have shown that the mitochondrial dysfunction and metabolic deficits in HD result from transcriptional dysregulation of peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α). As a transcriptional regulator, PGC-1α modulates several nuclear receptor transcription factors including PPARδ (Peroxisome Proliferator-Activated Receptor), the most abundantly expressed subtype in the central nervous system. Although its functional relevance in this tissue has not been well-defined, with the discovery that retinoic acid binds to PPARδ to mediate its previously well-documented pro-survival effect, PPARδ may be responsible for promoting a variety of survival / repair processes in neurons. PPARδ requires heterodimerization with retinoid X receptor (RXR) to bind its target genes. Bexarotene is a RXR selective agonist that activates PPARδ/RXR heterodimers. A recent study in a model of Alzheimer's disease (AD) highlighted the beneficial effects of bexarotene treatment in transgenic mice. Their data suggest that effectiveness in this AD model may be related to improved redox signaling, mitochondrial function, and protein processing as well as decreased inflammation and oxidative stress. Brain pathology of patients with Huntington's disease display similar deficits in redox signaling and mitochondrial function as well as increased inflammation and oxidative stress. We evaluated this promising therapeutic treatment, bexarotene, both *in vitro* in a full-length htt HD model cell line, and *in vivo*, in the N-terminal htt HD N171-82Q mouse model, which develops pathological features of HD. Our studies in primary cortical neurons from wild-type and Bacterial Artificial Chromosome Huntington's Disease (BAC-HD) mice indicate that modification of PPARδ signaling pathway components via bexarotene improves PPARδ transcriptional function, mitochondria function, and survival in primary cortical neurons. Bexarotene treatment in HD N171-82Q mice ameliorated kyphosis and motor symptoms (rotarod, ledge, clasping, gait), and overall slowed disease progression. We propose that bexarotene, in combination with a potent PPARδ agonist, is a promising approach to counter neuronal loss and motor symptom development in multiple neurodegenerative diseases including Huntington's disease.

Disclosures: **A.S. Dickey:** None. **E. Masliah:** None. **A.R. La Spada:** None. **N. Lomas:** None. **K.R. Sampat:** None. **D.N. Sanchez:** None.

Nanosymposium

769. Huntington's Disease Mechanisms and Therapeutic Strategies

Location: 147B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 769.10

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Department of Health RC14C

Title: PARP-1 inhibition is neuroprotective in the R6/2 mouse model of Huntington's disease

Authors: *F. R. FUSCO[1], A. CARDINALE[1], A. SERNANI[1], C. GIAMPÀ[1,2], G. BERNARDI[1,3];
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Abstract: Poly(ADP-ribose)polymerase 1 (PARP-1) is an abundant constitutive nuclear enzyme which is implicated in such physiological processes as DNA repair, genomic stability, and apoptosis. Moreover, PARP-1 has been shown to mediate necrotic cell death in response to excessive DNA damage under pathological conditions. A strong neuronal and glial expression of poly(ADP-ribose) polymerase (PARP)-immunoreactivity was observed in HD brains, suggesting the involvement of apoptosis in HD. In this study, we sought to determine if the PARP-1 inhibitor exerts a neuroprotective effect in R6/2 mutant mice, which recapitulates, in many aspects, human HD (Mangiarini et al, 1996). Transgenic mice were treated with the PARP-1 inhibitor INO-1001 mg/Kg daily starting from 4 weeks of age. After transcardial perfusion, histological and immunohistochemical studies were performed. We found that INO 1001- treated R6/2 mice survived longer and displayed less severe signs of neurological dysfunction than the vehicle treated ones. Moreover, R6/2 performed better on rotarod and open field tests compared to the vehicle treated ones. Primary outcome measures such as brain volume, striatal atrophy, size and morphology of striatal neurons, neuronal intranuclear inclusions and microglial reaction confirmed a neuroprotective effect of the compound. INO-1001 was effective in increasing significantly the levels of activated CREB and of BDNF the striatal spiny neurons, which might account for the beneficial effects observed in this model. Our findings show that PARP-1 inhibition could be considered as a valid therapeutic approach for HD.

Disclosures: F.R. Fusco: None. **A. Cardinale:** None. **A. Sernani:** None. **C. Giampà:** None. **G. Bernardi:** None.

Nanosymposium

769. Huntington's Disease Mechanisms and Therapeutic Strategies

Location: 147B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 769.11

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CIHR grant MOP-84438

ISIS Pharmaceuticals

Huntington Society of Canada

Michael Smith Foundation for Health Research

Title: *In vivo* evaluation of candidate allele-specific mutant Huntington gene silencing antisense oligonucleotide drugs

Authors: *A. L. SOUTHWELL[1], N. SKOTTE[1], H. KORDASIEWICZ[2], M. ØSTERGAARD[2], A. T. WATT[2], J. B. CARROLL[3], C. N. DOTY[1], E. B. VILLANUEVA[1], E. PETHOUKHOV[1], K. VAID[1], Y. XIE[1], S. M. FREIER[2], E. E. SWAYZE[2], P. P. SETH[2], C. F. BENNETT[2], M. R. HAYDEN[1];
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Abstract: Huntington disease (HD) is a dominant, genetic neurodegenerative disease characterized by progressive loss of voluntary motor control, psychiatric disturbance, and cognitive decline, for which there is currently no disease-modifying therapy. HD is caused by the expansion of a CAG tract in the huntingtin (HTT) gene. The mutant HTT protein (muHTT) acquires toxic functions, and there is significant evidence that muHTT lowering would be therapeutically efficacious. However, the wild-type HTT protein (wtHTT) serves vital functions, making allele-specific muHTT lowering strategies potentially safer than non-selective strategies. CAG tract expansion is associated with single nucleotide polymorphisms (SNPs) that can be targeted by gene silencing reagents such as antisense oligonucleotides (ASOs) to accomplish allele-specific muHTT lowering. We have evaluated ASOs targeted to HD-associated SNPs in acute in vivo studies including screening, distribution, duration of action and dosing, using a humanized mouse model of HD, Hu97/18, that is heterozygous for the targeted SNPs. We have identified four well-tolerated lead ASOs that potently and selectively silence muHTT at a broad range of doses throughout the central nervous system for 36 weeks or more after a single intracerebroventricular injection. We are currently conducting a preclinical therapeutic efficacy

trial of these lead ASOs and evaluating them for effect on the HD-like phenotypes of Hu97/18 mice. Treated mice are undergoing longitudinal behavioral and biochemical assessment followed by terminal electrophysiology or neuropathology. Contingent on findings from these studies and using delivery and dosing information gained from ongoing CNS ASO clinical trials, a primary SNP-targeted ASO drug could be fairly rapidly translated for human applications.

Disclosures: **A.L. Southwell:** None. **N. Skotte:** None. **H. Kordasiewicz:** A. Employment/Salary (full or part-time):; ISIS Pharmaceuticals. **M. Østergaard:** A. Employment/Salary (full or part-time):; ISIS Pharmaceuticals. **A.T. Watt:** A. Employment/Salary (full or part-time):; ISIS Pharmaceuticals. **J.B. Carroll:** None. **C.N. Doty:** None. **E.B. Villanueva:** None. **E. Pethoukhov:** None. **K. Vaid:** None. **Y. Xie:** None. **S.M. Freier:** A. Employment/Salary (full or part-time):; ISIS Pharmaceuticals. **E.E. Swayze:** A. Employment/Salary (full or part-time):; ISIS Pharmaceuticals. **P.P. Seth:** A. Employment/Salary (full or part-time):; ISIS Pharmaceuticals. **C.F. Bennett:** A. Employment/Salary (full or part-time):; ISIS Pharmaceuticals. **M.R. Hayden:** A. Employment/Salary (full or part-time):; Teva Pharmaceuticals.

Nanosymposium

769. Huntington's Disease Mechanisms and Therapeutic Strategies

Location: 147B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 769.12

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Engineered zinc finger transcriptional repressors selectively inhibit mutant huntingtin expression and reverse disease phenotypes in Huntington’s disease patient-derived neurons and in rodent models

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Abstract: Huntington’s disease (HD) is a fatal autosomal dominant neurodegenerative disease caused by CAG-trinucleotide repeat expansion in exon 1 of the Huntingtin (Htt) gene. Repeat lengths of 35 or fewer CAGs are not linked to pathophysiology, whereas 40 or more CAGs

invariably lead to HD, which most severely affects the basal ganglia and cerebral cortex and is characterized by a progressively worsening chorea, cognitive and psychiatric dysfunctions. Based on results from rodent studies that reduction of Htt levels lead to phenotypic improvement, multiple Htt-lowering strategies, such as RNAi and antisense oligonucleotides, are being pursued as potential therapies for HD. Most of these methods, either simultaneously down-regulate mutant Htt as well as wild type Htt, which may play critical roles in various cellular processes, or target mutant Htt based on single nucleotide polymorphisms (SNPs) that are only applicable to various subpopulations of of patients. Engineered zinc-finger protein transcription factors (ZFP TFs) can be designed to bind virtually any DNA sequence and regulate gene expression. By designing ZFPs to specifically recognize the CAG expansion, we sought to develop a therapeutic strategy that can selectively target the mutant Htt and be applied to the majority of the patient population. We showed that ZFPs can be engineered to minimally regulate the normal Htt alleles (CAG15-21) while driving approximately 90% repression of mutant Htt alleles (CAG40-69) in multiple fibroblasts lines derived from HD patients. Such allele-specific repression of mutant Htt can also be achieved in neurons differentiated from HD patient stem cells. Moreover, ZFP repressors of mutant Htt reversed multiple phenotypes exhibited by HD neurons, including reduced energetics and susceptibility to cell death upon growth factor withdrawal. To test their in vivo efficacy, ZFPs were delivered using adeno-associated virus (AAV) vectors to the striatum of HD model mice. In R6/2 mice, ZFPs led to significant reduced the levels of mutant Htt mRNA without affecting normal Htt levels; they also increased expression levels of medium spiny neuron markers, suggesting ZFP-mediated protection of those cells. Significant improvements in motor defects, such as clasping, were also observed in ZFP-treated R6/2 mice. In Q175 mice, ZFPs not only prevented mutant Htt aggregation when delivered at 2 months of age, but also led to significant clearance of existing Htt aggregates when delivered at 6 months of age. Together, these results support the further development of allele-specific ZFP repressors as a therapy for HD.

Disclosures: **H.S. Zhang:** A. Employment/Salary (full or part-time):; Sangamo BioSciences. **B. Zeitler:** A. Employment/Salary (full or part-time):; Sangamo BioSciences. **S. Froelich:** A. Employment/Salary (full or part-time):; Sangamo BioSciences. **Q. Yu:** A. Employment/Salary (full or part-time):; Sangamo BioSciences. **J. Pearl:** A. Employment/Salary (full or part-time):; Sangamo BioSciences. **D.E. Paschon:** A. Employment/Salary (full or part-time):; Sangamo BioSciences. **J.C. Miller:** A. Employment/Salary (full or part-time):; Sangamo BioSciences. **A.E. Kudwa:** A. Employment/Salary (full or part-time):; Psychogenics. **Y. Sedaghat:** A. Employment/Salary (full or part-time):; Evotec AG. **D. Li:** A. Employment/Salary (full or part-time):; Sangamo BioSciences. **K. Marlen:** A. Employment/Salary (full or part-time):; Sangamo BioSciences. **D. Guschin:** A. Employment/Salary (full or part-time):; Sangamo BioSciences. **L. Zhang:** A. Employment/Salary (full or part-time):; Sangamo BioSciences. **M. Mendel:** A. Employment/Salary (full or part-time):; Sangamo BioSciences. **E.J. Rebar:** A. Employment/Salary (full or part-time):; Sangamo BioSciences. **F.D. Urnov:** A. Employment/Salary (full or part-time):; Sangamo BioSciences. **S. Kwak:** None. **V. Mack:** A.

Employment/Salary (full or part-time):; Evotec AG. **I. Munoz-Sanjuan:** None. **P.D. Gregory:** A. Employment/Salary (full or part-time):; Sangamo BioSciences.

Nanosymposium

769. Huntington's Disease Mechanisms and Therapeutic Strategies

Location: 147B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 769.13

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Foundation

Title: Treating circadian dysfunction in a mouse model of Huntington's disease

Authors: R. E. FLORES[1], D. H. LOH[1], D. TRUONG[1], A. M. SCHROEDER[1], *C. S. COLWELL[2,1];
[1]Psychiatry and Biobehavioral Sci., Univ. of California - Los Angeles, Los Angeles, CA;
[2]UCLA, LOS ANGELES, CA

Abstract: Sleep and circadian disruption are a common complaint by patients with Huntington's disease (HD) and other neurodegenerative diseases. HD patients have trouble sleeping at night and exhibit excessive daytime sleepiness early in the disease progression. It is becoming increasingly clear that disruptions in sleep-wake and circadian rhythms result in a diverse set of symptoms including cardiovascular, metabolic, and cognitive dysfunction. We test the hypothesis that improvement of circadian rhythms in sleep/wake and activity can improve disease symptoms or delay disease progression in the BACHD mouse model of HD. This HD mouse model recapitulates the disease progression of HD including circadian rhythm disruption. We treated BACHD mice with in-phase and out-of-phase food intake, which results in altered circadian entrainment. Limiting food intake to the active phase reduced daytime activity and resulted in improved performance on motor coordination tests at the early symptomatic stage, confirming the value of strengthening the circadian system on the progression of HD.

Disclosures: R.E. Flores: None. **C.S. Colwell:** None. **D.H. Loh:** None. **D. Truong:** None. **A.M. Schroeder:** None.

Nanosymposium

770. Repeat Diseases From SBMA to Motor Neuron Disease

Location: 146C

Time: Wednesday, November 19, 2014, 1:00 PM - 3:00 PM

Presentation Number: 770.01

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: F31-NS-077792-03

Title: Preventing the n/c interaction of the androgen receptor delays disease in a mouse model of SBMA

Authors: *L. J. COOPER, D. CURTIS, D. MERRY;
Biochem. and Mol. Biol., Thomas Jefferson Univ., Philadelphia, PA

Abstract: Spinal and bulbar muscular atrophy (SBMA) is a neurodegenerative disease caused by polyglutamine expansion in the androgen receptor (AR) and is associated with misfolded and aggregated species of the mutant AR. We have recently shown that the interaction between the amino-terminal FxxLF motif and carboxyl-terminal AF-2 domain (N/C interaction) that occurs in the AR upon DHT binding is necessary for toxicity and AR aggregation. Preventing this interaction, by mutating the FxxLF motif, ameliorated aggregation and toxicity in a cell model of SBMA. Additionally, we recently found that motor neurons overexpressing polyglutamine-expanded AR with a mutated FxxLF motif did not exhibit DHT-dependent toxicity, whereas overexpression of polyglutamine-expanded AR with an intact N/C interaction was toxic to motor neurons. To investigate the role of this interdomain interaction in vivo, we created transgenic mice (ARF23A108Q) that express polyQ-expanded AR with a mutation in the FxxLF motif (F23A) to prevent the N/C interaction. ARF23A108Q mice express AR at a slightly higher level than N/C-intact SBMA mice but showed a reduction in aggregation of mutant AR in the spinal cord and brain. Male ARF23A108Q mice had delayed onset of both rotarod and balance beam deficits, compared to male SBMA mice. These results suggest that preventing the N/C interaction delays disease onset in mice and could be a useful therapeutic target in patients with SBMA.

Disclosures: L.J. Cooper: None. **D. Curtis:** None. **D. Merry:** None.

Nanosymposium

770. Repeat Diseases From SBMA to Motor Neuron Disease

Location: 146C

Time: Wednesday, November 19, 2014, 1:00 PM - 3:00 PM

Presentation Number: 770.02

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: FEDER through through Programa Mais Centro (CENTRO-07-ST24-FEDER-002002, 002006, 002008)

Competitive Factors Operational Program – COMPETE

Portuguese Foundation for Science and Technology, PTDC/SAU-NEU/099307/2008, PTDC/SAU-NMC/116512/2010 and PEst-C/SAU/LA0001/2013-2014

Richard Chin and Lily Lock Machado-Joseph research fund

National Ataxia Foundation

Portuguese Foundation for Science and Technology, fellowships SFRH/BPD/72507/2010 and SFRH/BPD/62945/2009

Title: Transplantation of cerebellar neural stem cells improves motor coordination and neuropathology in a transgenic mouse model of Machado-Joseph disease

Authors: L. MENDONÇA[1], C. NOBREGA[1], H. HIRAI[2], B. KASPAR[3], *L. PEREIRA DE ALMEIDA[1,4];
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Abstract: Machado-Joseph disease is a neurodegenerative disease without effective treatment. Machado-Joseph disease patients exhibit significant motor impairments such as gait ataxia, associated to multiple neuropathological changes including mutant ataxin-3 inclusions, marked neuronal loss and atrophy of the cerebellum. Thus, an effective treatment of symptomatic Machado-Joseph disease patients may require cell replacement. Therefore in this work, we investigated whether transplantation of cerebellar neural stem cells (cNSC) into the cerebellum of adult Machado-Joseph disease transgenic mice would alleviate neuropathological, neuroinflammatory, neurotrophic factor levels and motor coordination. We found that upon transplantation into the cerebellum of adult Machado-Joseph disease mice, cNSC differentiated into neurons, astrocytes and oligodendrocytes. Importantly, cNSC transplantation mediated a significant and robust alleviation of the motor behavior impairments, which correlated with a preservation from Machado-Joseph disease associated neuropathology, namely reduction of

Purkinje cells loss, reduction of cellular layers shrinkage and mutant ataxin-3 aggregates. Additionally, a significant reduction of neuroinflammation and an increase of neurotrophic factors levels were observed, indicating that transplantation of cNSC also trigger important neuroprotective effects. Thus, cNSC have the potential to be used as a cell replacement and neuroprotective approach for Machado-Joseph disease therapy.

Disclosures: L. Mendonça: None. **C. Nobrega:** None. **H. Hirai:** None. **B. Kaspar:** None. **L. Pereira de Almeida:** None.

Nanosymposium

770. Repeat Diseases From SBMA to Motor Neuron Disease

Location: 146C

Time: Wednesday, November 19, 2014, 1:00 PM - 3:00 PM

Presentation Number: 770.03

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Drug repositioning for Friedreich's ataxia identifies 'unsilencers' of frataxin, that work in cell and animal models

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Abstract: Friedreich's ataxia (FA) is caused by an inherited deficiency in the mitochondrial protein frataxin, which results in neurodegeneration of Dorsal Root Ganglia and spinocerebellar tracts. There is no approved therapy for FA. Frataxin deficiency results in a decrease in iron-sulfur cluster biogenesis and antioxidant defense. We identified a thiol-based redox deficiency in FA cells and animal models, and screened a 1600-compound library of FDA-approved drugs, to identify whether these might have beneficial effects in FA cell and animal models. The anesthetic dyclonine increased frataxin transcript and protein dose-dependently in FA cells and brains of animal models. Dyclonine rescued frataxin-dependent enzyme deficiencies in the iron-sulfur enzymes aconitase and succinate dehydrogenase. We have identified multiple neurobehavioral deficits in two animal models of FA, including beam walking, Treadscan and Von Frey, and functionally, dyclonine rescued some of these deficits, namely beam-walking. A human clinical proof-of-concept study was completed in eight Friedreich's ataxia patients dosed twice daily using a 1% dyclonine rinse for one week. Six of eight patients showed an increase in buccal cell frataxin levels, and fold induction was significantly correlated with disease severity. Mechanistically, dyclonine induces the Nrf2 transcription factor, which we show binds an upstream response element in the frataxin locus. Dyclonine also inhibited the activity of histone

methyltransferase G9a, known to methylate histone H3K9 to silence Friedreich's chromatin. Dyclonine represents a novel therapeutic strategy that can potentially be repurposed for the treatment of FA.

Disclosures: G. Cortopassi: None. **M. Jasoliya:** None. **S. Perlman:** None. **S. Sahdeo:** None.

Nanosymposium

770. Repeat Diseases From SBMA to Motor Neuron Disease

Location: 146C

Time: Wednesday, November 19, 2014, 1:00 PM - 3:00 PM

Presentation Number: 770.04

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: R01NS033123

R21NS081182

RC4NS073009

Title: MOE antisense oligonucleotides for the treatment of spinocerebellar ataxia type 2 (SCA2)

Authors: *D. R. SCOLES[1], M. D. SCHNEIDER[1], B. HENRIE[1], G. HUNG[2], C. F. BENNETT[2], S. M. PULST[1];
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Abstract: Spinocerebellar ataxia type 2 (SCA2) is an autosomal dominant inherited disorder caused by CAG repeat expansion in the *ATXN2* gene. The *ATXN2* gene protein product ataxin-2 is characterized by gains of function upon *ATXN2* mutation, caused by expansion of the CAG-encoded polyglutamine tract in ataxin-2. We hypothesize that lowering overall *ATXN2* expression using antisense oligonucleotides (ASOs) would be therapeutic for SCA2. This hypothesis is supported by the lack of neurodegeneration in *ATXN2* knockout mice, and inducible reversibility of polyQ disease mouse phenotypes of HD and SCA1. **Objective:** To develop a molecular approach for lowering *ATXN2* expression toward the development of a treatment for SCA2. **Methods:** We screened 153 ASOs designed to lower *ATXN2* expression *in vitro* in HepG2 cells. We evaluated the most efficacious ASOs for lowering *ATXN2* expression in two SCA2 mouse models. Expression of *ATXN2* and *Iba1* was evaluated by quantitative PCR, Ataxin-2 expression was determined by immunoblotting, and ASO localization was evaluated by immunostaining. ASO effects on the SCA2 mouse motor phenotype were determined using the accelerating rotarod. **Results:** IC50s for lowering *ATXN2* expression *in vitro* were determined

for the 15 best ASOs, and the top 8 leads were advanced. ASO efficacy for lowering both human *ATXN2* and mouse *Atxn2* expression, and for activating the microgliosis marker *Iba1* was determined *in vivo* by intracerebroventricular injection in transgenic *ATXN2-Q127* mice and BAC-*ATXN2* mice. Immunohistochemical stains using anti-ASO antibody demonstrated ASOs localized in Purkinje cells and cells of the deep cerebellar nuclei. The top 3 lead ASOs for lowering *ATXN2* expression without elevating *Iba1* were evaluated for modifying the SCA2 mouse rotarod phenotype of transgenic *ATXN2-Q127* mice. Each of the top three lead ASOs significantly inhibited further deterioration of the SCA2 mouse motor phenotype, measured after 10 weeks treatment (bolus doses of 100-200 μg). **Conclusions:** We identified three lead ASOs that lower *ATXN2* expression without elevating *Iba1* expression. The ASOs significantly inhibited further deterioration of the SCA2 mouse motor phenotype.

Disclosures: D.R. Scoles: None. **M.D. Schneider:** None. **B. Henrie:** None. **G. Hung:** None. **C.F. Bennett:** None. **S.M. Pulst:** None.

Nanosymposium

770. Repeat Diseases From SBMA to Motor Neuron Disease

Location: 146C

Time: Wednesday, November 19, 2014, 1:00 PM - 3:00 PM

Presentation Number: 770.05

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Farber Family Foundation

Title: RAN dipeptides linked to C9ORF72-ALS/FTD form toxic nuclear aggregates and cause *in vitro* and *in vivo* neuronal death

Authors: *X. WEN[1], W. TAN[1], K. KRISHNAMURTHY[1], J. MONAGHAN[2], U. B. PANDEY[2], P. PASINELLI[1], D. TROTTI[1];
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Abstract: Expansion of GGGGCC (G4C2) hexanucleotide repeats located in the non-coding region of C9ORF72 is the most common cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Unaffected individuals usually carry up to 23 G4C2 repeats, whereas affected individuals carry up to hundreds repeats. Di-peptide repeat proteins (DRPs) resulted from repeat-associated non-ATG initiated (RAN) translation were seen in C9-ALS/FTD

patients. To test whether DRPs contribute to disease pathogenesis, constructs expressing RAN DRPs were designed. Primary cortical and motor neuron models were used. Longitudinal imaging analysis was performed to determine the toxicity of each construct in primary neuronal models. Hazard function of each construct was deduced from Kaplan-Meier survival analysis. We found that Proline-Arginine (PR) DRP is the most neurotoxic species, which was confirmed also in vivo in Drosophila. Evidence suggested that PR-induced toxicity correlates with the formation of nuclear aggregates. PR DRPs aggregate in nucleoli and cause a stress-response in cells, with a reduction in the number of processing bodies and formation of stress granules. Furthermore, G4C2-neurotoxicity is independent from RAN translated products, although the toxic effect synergizes with that of PR-aggregates, suggesting a convergence of mechanisms.

Disclosures: X. Wen: None. **W. Tan:** None. **K. Krishnamurthy:** None. **J. Monaghan:** None. **U.B. Pandey:** None. **P. Pasinelli:** None. **D. Trotti:** None.

Nanosymposium

770. Repeat Diseases From SBMA to Motor Neuron Disease

Location: 146C

Time: Wednesday, November 19, 2014, 1:00 PM - 3:00 PM

Presentation Number: 770.06

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH GRANT NS062051

NIH GRANT NS082351

National Ataxia Foundation

Brain Research Foundation

Title: Testing the role of histone deacetylase HDAC3 in Purkinje cell function in the context of Spinocerebellar ataxia type 1 using a genetic approach

Authors: *P. OPAL[1], Y.-S. HU[2], A. VENKATRAMAN[2], A. DIDONNA[2], M. CVETANOVIC[2], A. KRBANJEVIC[2], P. BILESIMO[2];
[1]Dept Neurol., Northwestern Univ. Med. Sch., CHICAGO, IL; [2]Northwestern Univ., Chicago, IL

Abstract: Spinocerebellar ataxia type 1 (SCA1) is a relentless neurodegenerative disease caused by a pathogenic glutamine repeat expansion in the protein ataxin-1 (ATXN1). We have found that one mechanism mediating pathogenesis is excessive transcriptional repression induced by

mutant ATXN-1. ATXN1 binds HDAC3, a class I histone deacetylase that is required for ATXN1-induced transcriptional repression. We therefore tested whether depleting HDAC3 improves the phenotype of the SCA1 knock-in mouse (SCA1154Q/2Q), a very precise model of SCA1. For these studies we used a genetic approach. Moreover, because HDAC3 null mice are embryonic-lethal, we used for our analyses a combination of HDAC3 haplo-insufficient and Purkinje cell-specific HDAC3 conditional knock-out mice. We found that although deleting a single allele of HDAC3 in the context of SCA1 was insufficient to improve cerebellar and cognitive deficits of the disease, a complete loss of Purkinje cell HDAC3 was highly deleterious to the normal functioning of Purkinje neurons. From these findings, we conclude that although pharmacological inhibition of HDAC3 may yet have a role in SCA1 therapy, depleting HDAC3 activity could produce untoward effects in the central nervous system. Thus the neurotoxic consequences of HDAC3 depletion should be considered wherever pharmacologic inhibition of HDAC3 is being contemplated for neurodegeneration or indeed other disorders. Understanding the transcriptional networks regulated by HDAC3 in the brain will be an important issue to address in future studies.

Disclosures: P. Opal: None. **Y. Hu:** None. **A. Venkatraman:** None. **A. Didonna:** None. **M. Cvetanovic:** None. **A. Krbanjevic:** None. **P. Bilesimo:** None.

Nanosymposium

770. Repeat Diseases From SBMA to Motor Neuron Disease

Location: 146C

Time: Wednesday, November 19, 2014, 1:00 PM - 3:00 PM

Presentation Number: 770.07

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Reduced inositol 1, 4, 5-triphosphate receptor type 1 and carbonic anhydrase 8 gene expression is a pathway for cerebellar degeneration

Authors: *K. ISHIKAWA;
Dept. of Neurol., Tokyo Med. and Dent. Univ., Tokyo, Japan

Abstract: Background and Aim: Cerebellar degeneration in human is a group of heterogeneous neurodegenerative conditions. Although a variety of genes cause cerebellar degenerations, it is not clear whether a certain molecular alteration exists in these degenerative conditions. Identifying the common pathway(s) is important not only for our understanding these conditions, but also for searching potential therapies. We undertook gene expression analysis on human cerebellar tissues using microarray. Materials and Methods: Four subjects affected with

spinocerebellar ataxia type (SCA) 6 (n=2) or SCA31 (n=2), both of which cause cerebellar cortical degenerations, were studied. For control, eight male subjects ranging in age from 57 to 89 years were studied. None of these individuals had neurological diseases. From all individuals, brains were removed at autopsy, and routine histological examinations excluded presence of abnormal neuropathologic changes such as cerebral infarct or senile plaques. The right halves of the brains were frozen as soon as possible after the autopsy, and had been kept frozen until use. Tissue sections from the upper vermis ("V"), upper hemisphere ("UH") and lower hemisphere ("LH") were obtained from each individual, and RNA was extracted separately. The gene expression was investigated using Affymetrix Human Total Exon microarray. In each of the three cerebellar regions (i.e., V, UH, LH), we compared the changes of gene expressions between SCA subjects and controls using a statistical algorithm MAS5. For each region of cerebellum, genes that showed greater than 2 fold increase or those showing less than 0.5 were both considered as the genes significantly altered in the SCA cerebella compared to that of control cerebella. Then, we searched for mRNAs that consistently showed expression changes in three regions. Selected mRNAs were validated by quantitative reverse-transcription PCR (qRT-PCR). Results: Multiple probes were found decreased in SCA samples. Interestingly, mRNA for inositol 1,4,5-trisphosphate receptor, type 1 gene (ITPR1), which is the cause of SCA15, was reduced in SCA6 and SCA31 cerebellar tissues. The mRNA for carbonic anhydrase 8 (CA8), which is the ligand of ITPR1 and the cause of autosomal recessive cerebellar ataxia in human, was also decreased. These findings suggest that ITPR1-CA8 pathway is down-regulated in two SCAs not causally related to the two molecules. Conclusions: Reduced ITPR1-CA8 pathway may be involved in various cerebellar degenerations.

Disclosures: K. Ishikawa: None.

Nanosymposium

770. Repeat Diseases From SBMA to Motor Neuron Disease

Location: 146C

Time: Wednesday, November 19, 2014, 1:00 PM - 3:00 PM

Presentation Number: 770.08

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: R21 NS084528-1 NINDS

Title: Adeno-associated virus mediated C9orf72 expanded GGGGCC repeat expression causes both behavioral and motor impairments in mouse model of FTD/ALS

Authors: *J. CHEW[1,2], H. SASAGURI[2], K. JANSEN-WEST[2], W. LEE[2], A. KURTI[2], J. FRYER[1,2], L. PETRUCELLI[1,2];
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Abstract: The *C9orf72* hexanucleotide repeat expansion (GGGGCC) has been identified as the major genetic cause underlying both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) in 2011, yet the molecular pathogenesis of this expanded repeat remains unknown. To date, there has been no report of a mouse model expressing this pathological repeat expansion that successfully recapitulates the characteristic behavioral/motor deficits associated with c9FTD/ALS. Our preliminary results showed that adeno-associated viral (AAV)- mediated transduction of an expanded number of GGGGCC repeats in mouse primary neurons resulted in accumulation of nuclear RNA foci detected by RNA fluorescent-in-situ hybridization (FISH) and repeat- associated non-ATG (RAN) translated polypeptides which were absent when expressing non-pathogenic number of repeats. We therefore developed a novel mouse model expressing an expanded GGGGCC repeat or a non-pathological repeat length RNA mediated by AAV transduction into the neurons of the central nervous system (CNS) of neonatal mice through intracerebroventricular (ICV) administration. After 6 months of age, these mice were subjected to a battery of behavioral and motor performance tests. Mice expressing an abnormal number of repeats exhibited both behavioral and motor abnormalities when compared to mice expressing non-pathogenic repeats. In addition, affected mice also had a decreased brain weight compared to controls. Therefore these important data suggest that the expression of expanded repeats in the CNS can recapitulate some of c9FTD/ALS-like phenotypes and that further characterization of this mouse model will be a useful tool in elucidating the pathogenic role of expanded repeat expression in c9ALS/FTD.

Disclosures: J. Chew: None. **H. Sasaguri:** None. **K. Jansen-West:** None. **W. Lee:** None. **A. Kurti:** None. **J. Fryer:** None. **L. Petrucelli:** None.

Nanosymposium

771. Stroke Recovery

Location: 152B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 771.01

Topic: C.21.Stroke Recovery

Title: Blocking gap junctions halts the spread of cortical spreading depression; a computational study

Authors: *M. A. CHARY, S. MAYER;
Mount Sinai Sch. of Med., New York, NY

Abstract: Cortical spreading depression (CSD) is a wave of failure of ion homeostasis that interrupts normal brain functions. It can occur spontaneously or after injury. When CSD occurs after injury, it increases the amount of irreversibly damaged tissue. Blocking NMDA receptors halts cortical spreading depression in animal models. This suggests that excitotoxicity is important in the propagation of cortical spreading depression. Gap junctions between neurons are required for NMDA receptor-mediated excitotoxicity. However, the role of gap junctions in cortical spreading depression remains largely unexplored. Here use a computational model to demonstrate that blocking gap junctions increases the number of CSD waves but decreases the penetrance of those waves. Our results suggest that gap junctions play an important role in cortical spreading depression. We hope that our computational model will motivate more detailed computational models and experiments to better understand the role of chemical synapses in damage after neuronal injury. More broadly, blocking gap junctions may provide an alternative and even more specific target to limit damage after neuronal injury.

Disclosures: M.A. Chary: None. **S. Mayer:** A. Employment/Salary (full or part-time):; Mount.

Nanosymposium

771. Stroke Recovery

Location: 152B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 771.02

Topic: C.21.Stroke Recovery

Support: COLCIENCIAS. Code project: 111551928905 (2011-2013)

Title: Prevention of pathophysiology synaptic hippocampal by stroke: cdk5 a therapeutic target

Authors: *J. A. GUTIERREZ VARGAS, G. CARDONA-GÓMEZ;
Univ. of Antioquia/ Group of Neurosci., Medellin, Colombia

Abstract: CDK5 is a kinase involved in synaptic plasticity; its dysregulation contributes to tauopathy and consequent neurodegeneration. In this study we evaluated the CDK5 silencing effect at short and long term post-ischemia on cognitive function, tauopathy and synaptic plasticity pathways. Wistar rats were subjected to brain ischemia (t-MCAO) and they were injected with shCDK5miR (CDK5 interfering version) or shSCRmiR (control version) in the

hippocampus during the occlusion of the middle cerebral artery. Learning and spatial memory were assessed at 1 month and 4 months post-ischemia, subsequently were performed the histological and biochemical analysis to short and long-term post-ischemia respectively. Our results show that silencing of CDK5 to short-term in ischemic animals improving the deficit in learning, memory, and re-learning. These are supported by a reversal marker of tauopathy, as increased neurotrophin BDNF and TrkB receptor and its transcriptional activator CREB. Improved cognition by silencing of CDK5 is maintained for long term post-ischemia, being supported by the prevention of tauopathy, increased of BDNF and activation plasticity routes associated with memory and learning. Our results suggest that silencing of CDK5 to short term reverses tauopathy and avoid cognitive impairment; this protection is maintained to long term post-ischemia through increased trophic factors (BDNF) and activation of survival pathways and synaptic plasticity.

Disclosures: J.A. Gutierrez Vargas: None. **G. Cardona-Gómez:** None.

Nanosymposium

771. Stroke Recovery

Location: 152B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 771.03

Topic: C.21.Stroke Recovery

Support: NIH grant NS0458710

NIH grant R41NS073378

NIH grant NS057255

NIH grant NS075338

NIH grant NS062097

AHA12GRNT12060222

Title: Sensorimotor and social behavioral benefits of intranasally delivered bone marrow mesenchymal stem cells after neonatal stroke in rats

Authors: *Z. WEI[1], X. GU[1], J. LEE[1], S. YU[1], L. WEI[1,2];
[1]Anesthesiol., [2]Neurol., Emory Univ., Atlanta, GA

Abstract: Neonatal stroke is a major cause of mortality and long term morbidity in infants and children. Currently very limited therapeutic strategies are available to protect the developing brain against ischemic damage and/or promote brain repair for pediatric patients. Cell-based transplantation using bone marrow mesenchymal stem cells (BMSCs) has emerged as a regenerative therapy after stroke. In the present investigation, we applied a non-invasive and brain targeted intranasal administration of BMSCs in a neonatal rat focal ischemia model and focused on behavioral/functional recovery after stroke. All BMSCs received hypoxic-preconditioning before transplantation to enhance their tolerance and regenerative properties. BMSCs or vehicle control were then delivered intranasally 6 hrs and 3 days after stroke. Our results showed that BMSC administration attenuated the reduction of cerebral blood flow and stimulated neurogenesis and angiogenesis in stroke neonates. In adhesive removal test and buried food finding tests performed 2 weeks after transplantation, BMSC-treated rats showed better sensorimotor and olfactory functional recovery than saline-treated animals. Social behaviors that were evaluated using a social interaction test and a social novelty test were significantly improved in BMSC-treated animals. Overall, the non-invasive transplantation therapy using hypoxic-preconditioned BMSCs shows great potential as a regenerative treatment during the sub-acute and chronic phases after neonatal stroke and may improve long-term recovery in sensorimotor and social behavioral activities.

Disclosures: **Z. Wei:** None. **X. Gu:** None. **J. Lee:** None. **S. Yu:** None. **L. Wei:** None.

Nanosymposium

771. Stroke Recovery

Location: 152B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 771.04

Topic: C.21.Stroke Recovery

Support: NIH grant NS0458710

NIH grant R41NS073378

NIH grant NS057255

NIH grant NS075338

NIH grant NS062097

AHA GRNT12060222

Title: Transplantation of human iPS cell-derived neural progenitor cells improves regeneration and functional recovery after ischemic stroke in neonatal rats

Authors: D. CHEN[1], Z. WEI[1], J. LEE[1], X. GU[1], S. YU[1], *L. WEI[1,2];
[1]Dept. of Anesthesiol., [2]Neurol., Emory Univ., Atlanta, GA

Abstract: Neonatal stroke has been recognized as a significant cause of mortality and long-term neurological deficits in infants with very limited therapeutic options. As a promising regenerative therapy to replace or repair damaged tissue after stoke, human induced pluripotent stem (hiPS) cells transplantation has been shown to improve the regeneration after ischemic stroke in adult animals. Whether hiPS cell transplantation could provide therapeutic benefits in neonatal stroke is still unknown. In the present investigation, vector-free and transgene-free hiPS cells (iPS-DF19-9/7T) were differentiated to neural progenitors and subjected to hypoxic preconditioning before transplantation. Postnatal day 7 rats were subjected to focal ischemia targeting the right sensorimotor cortex. hiPS-derived neuroprogenitor cells (hiPS-NPCs) were transplanted to the penumbra and core region 7 days after stroke. The preconditioned cells survived well in the ischemic brain 3 days after transplantation. The cell transplantation increased the number of Glut-1+/BrdU+ cells and NeuN+/BrdU+ cells in the penumbra region compared to stroke controls. Using a home cage monitoring system, we showed that stroke animals that received hiPS-NPC transplantation were more active and slept better. Their sensorimotor function recovered better in the corner test (more equal left and right turns). In social behavior tests these animals showed improved social interactions with stranger rats compared to stroke controls. No tumor formation was seen up to 12 months after transplantation. These data suggest that vector-free and transgene-free hiPS cells are an ideal source for stem cell transplantation therapy that can stimulate regenerative mechanisms and improve the functional recovery after neonatal stroke.

Disclosures: D. Chen: None. **L. Wei:** None. **Z. Wei:** None. **J. Lee:** None. **X. Gu:** None. **S. Yu:** None.

Nanosymposium

771. Stroke Recovery

Location: 152B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 771.05

Topic: C.21.Stroke Recovery

Support: CIRM Grant RT2-01881

NIH Grant R01NS079691

Title: Biopolymer hydrogels promote neural precursor cell survival as a transplanted matrix in stroke

Authors: *P. MOSHAYEDI[1], A. R. BERG[1], J. P. LAM[2], L. NIH[2], W. LOWRY[3], T. SEGURA[2], S. T. CARMICHAEL[1];
[1]Dept. of Neurol., [2]Dept. of Chem. and Biochem., [3]Dept. of Mol. Cell and Developmental Biol., Univ. of California Los Angeles, Los Angeles, CA

Abstract: Stroke is a leading cause of disability in the world. Neural stem or progenitor cell transplantation in stroke has been shown to induce repair and recovery. However, cell transplantation in stroke is limited by poor transplant survival. Here we have employed self-polymerizing hyaluronan (HA)-based gels to encapsulate neural precursor cells (NPCs) upon transplantation into a cortical model of brain infarction. Hydrogel parameters such as elasticity, cross-linking characteristics and incorporation of growth factors or motifs for proteins commonly found in precursor cell-extracellular matrix interactions are crucial in tissue reconstruction. Examining a range of elasticities revealed that injecting a hydrogel of 350 Pa, which is close in elasticity to that of the brain, will promote the best replacement of infarcted tissue but imposes no stress on surrounding brain, as measured by gliosis and inflammation. By using different crosslinkers HA gels can be rendered sensitive or resistant to matrix metalloproteinases (MMPs) enzymes that are active in stroke and might interact with the HA matrix. This study revealed that MMP-sensitive HA gels promote angiogenesis by 9-folds; possibly because MMP-secreting endothelial cells are able to digest their way up in the gel matrix. We further enriched HA gels by incorporating motifs of extracellular matrix (ECM) proteins and growth factors. To extend these capabilities to cell transplantation approaches, human NPCs derived from induced-pluripotent stem (iPS) cells were encapsulated into HA gels with varying concentrations of RGD, YIGSR and IKVAV motifs (from fibronectin and laminin molecules) as well as BMP4 and BDNF. Subsequently the optimized combination that promoted the highest cell survival in vitro was discovered. This finding was confirmed when the optimized gel induced the highest proliferation of encapsulated cells in vivo. In addition, the same combination of HA gel promoted the highest rate of neuronal differentiation in encapsulated cells, as well as angiogenesis and integration with astrocytic backbone of the host tissue. We then showed that encapsulation in optimized HA gel improves survival of cells upon transplantation into cortical infarcts of drug-induced immunosuppressed wild-type mice. These preliminary results show HA-based biopolymers are a viable method of NPC delivery to infarcted brain and they can also promote cells survival once they are supplemented with optimized combination of growth factors and motifs of proteins present in neural precursor cell niche. Future studies will now focus to discover any advantage for HA-based hydrogels to improve motor deficits after cortical stroke.

Disclosures: **P. Moshayedi:** None. **A.R. Berg:** None. **J.P. Lam:** None. **L. Nih:** None. **W. Lowry:** None. **T. Segura:** None. **S.T. Carmichael:** None.

Nanosymposium

771. Stroke Recovery

Location: 152B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 771.06

Topic: C.21.Stroke Recovery

Support: NIH R25

Title: The effect of kappa opioid receptor activation following cerebral ischemia

Authors: S. HOANG[1], *E. WANG[2], M. CHENG[2], G. STEINBERG[2];
[1]Stanford Univ., STANFORD, CA; [2]Dept. of Neurosurg., Stanford Univ., Stanford, CA

Abstract: Kappa opioid receptor is an inhibitory G protein-coupled receptor through which endogenous dynorphins regulate physiological functions. Kappa (k)-opioid receptor (KOR) activation has been shown to attenuate brain injury in models of global and focal cerebral ischemia. Functional recovery following cerebral ischemia is mediated partly by angiogenesis and vascular reorganization. During development, the kappa opioid system has been shown to inhibit vascular development through endothelial cell differentiation and vascular pathfinding. KOR knockout mice showed a significant increase in overall vascular formation and ectopic vascular invasion into somites during embryonic development. However, despite KOR's role in neuroprotection, its role in recovery and angiogenesis is unclear. In this study, we examined the expression profile of the kappa opioid receptor and its ligand dynorphin following stroke, and investigated its role in angiogenesis and functional recovery. Focal cerebral ischemia was induced in adult male C57BL/6J mice at 8-12 weeks and mice were sacrificed at various time points. Immunohistochemistry was used to examine the expression profile of kappa opioid receptor and its ligand, dynorphin. Immunohistochemical analysis indicated that KOR expression was upregulated as early as day 1 post-stroke and continued to be elevated at 1 week post-stroke. The KOR up-regulation was restricted to the ischemic core with a neuronal morphology. Interestingly, there was no significant change of dynorphin expression in the ischemic core. Our preliminary results indicate that the kappa opioid receptor is significantly upregulated after stroke, whereas the expression of its ligand, dynorphin, remains unchanged. Current studies will examine the effect of blocking the kappa opioid receptor activation through pharmacological and optogenetics methods to determine its role in in angiogenesis and recovery post-stroke.

Disclosures: S. Hoang: None. **E. Wang:** None. **M. Cheng:** None. **G. Steinberg:** None.

Nanosymposium

771. Stroke Recovery

Location: 152B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 771.07

Topic: C.21.Stroke Recovery

Support: CIHR TPRM

Ontario Government Scholarship

Centre for Stroke Recovery

Heart and Stroke Foundation

Title: Endogenous neural stem cell stimulation with locally delivered cyclosporine

Authors: *A. TULADHAR[1], C. M. MORSHEAD[1,2], M. S. SHOICHET[1,3,4];
[1]Inst. of Biomaterials and Biomed. Engin., [2]Dept. of Surgery, [3]Dept. of Chem. Engin. and Applied Chem., [4]Dept. of Chem., Univ. of Toronto, Toronto, ON, Canada

Abstract: Each year approximately 5 million people are left with permanent disabilities due to stroke, with no treatments currently available to regenerate the lost brain tissue. Pharmacological stimulation of endogenous neural stem and progenitor cells (NSPCs) has been identified as a promising therapeutic approach for regeneration. However, many promising drug treatments (e.g. BDNF, GDNF, cyclosporine) are hindered by poor blood-brain barrier penetration or invasive delivery methods. We have previously reported the use of a bioactive hydrogel made of hyaluronan and methylcellulose (HAMC) for local release via epi-cortical drug delivery (Wang et al, Biomaterials, 2013; Caicco et al, J Control Release, 2013). Herein we evaluate the ability of locally released cyclosporine (CsA) to stimulate endogenous NSPCs and reduce tissue damage after stroke. We measured the effect of local CsA delivery on NSPCs in the lateral ventricles of uninjured rats and rats given a focal ischemic stroke using endothelin-1. Local release was achieved using CsA-loaded poly(lactic-co-glycolic acid) microparticles dispersed in a HAMC hydrogel. This delivery system was injected onto the surface of the brain using a minimally invasive procedure and the brains were harvested for immmunohistochemistry analysis 7 days after implantation. NSPC activity was measured by $Ki67^+$ staining in the lateral ventricles and stroke infarct was measured by $NeuN^-$ staining regions in the brain. Two-way ANOVA revealed a significant effect of local CsA delivery in the contralateral hemisphere, with an increase in NSPC proliferation in stroke-injured animals (1.2x fold increase). There was no significant effect of injury in the contralateral hemisphere. In the ipsilateral hemisphere there was no significant

effect of CsA delivery. However, there was a significant effect of injury that may have masked the effects of CsA. Stroke infarct was reduced in both CsA and vehicle treated animals compared to animals that received no treatment, revealing a protective effect of the HAMC hydrogel alone. This is likely due to the anti-inflammatory effects of hyaluronan (Wang et al, Biomaterials, 2012). The HAMC hydrogel provided a neuroprotective effect 7 days after stroke. Local delivery of CsA had a significant effect on NSPC activity in the contralateral hemisphere, but not in in the ipsilateral hemisphere. Future studies will focus on the effects of CsA in the chronic period after stroke (≥14 days) and combinatory effects with other neuroregenerative molecules.

Disclosures: **A. Tuladhar:** None. **C.M. Morshead:** None. **M.S. Shoichet:** None.

Nanosymposium

771. Stroke Recovery

Location: 152B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 771.08

Topic: C.21.Stroke Recovery

Support: NIH Grant

Title: Increase of finger flexion pressure with increased shoulder abduction loading during hand open in individuals with severe stroke

Authors: ***Y. LAN**, J. YAO, J. DEWALD;
Northwestern Univ., Chicago, IL

Abstract: Introduction Stroke is reported to be the primary cause of adult disability in the United States. One of the most pronounced signs in the majority of the stroke population is the inability to open the paretic hand. Finger extension is limited, and sometimes hand closure is even observed during a hand-opening attempt. Therefore, the objective of this study is to investigate the finger behavior during a hand opening in individuals with severe stroke, and how this behavior is affected by shoulder abduction loading. Material and Method A total of five individuals with severe stroke (Chedoke hand =2-3 out of 7, Fugl-Meyer score=22±3 out of 66) and five age-matched able-bodied individuals were recruited for this study. During the experiment, subjects were seated in a Biodex chair, and their test arm was placed at 75° of shoulder abduction, 45° of shoulder flexion and 90° of elbow flexion. Their thumb and fingers were rested on a cylinder wrapped around by a TactArray pressure sensor mat (Pressure Profile Systems, Inc., Los Angeles, California, USA). Upon the start of each trial, subjects were

instructed to perform a forward reach resulting in 90° shoulder flexion and full elbow extension, followed by opening the hand with a maximal effort during three conditions: 1) a fully supported arm; 2) lifting the arm with 25% of maximum shoulder abduction (SABD) torque; 3) lifting the arm with 50% of maximum SABD torque. Pressure generated at each fingertip area was calculated and normalized by the largest value across all trials. Results The main finding of this study are 1) One-way ANOVA showed that SABD loads had a significant impact on such finger flexion pressure ($F=5$, $p<0.05$) . The finger flexion pressure was increased with SABD loading. A paired-samples t-test showed a significant difference of loading on thumb ($p<0.05$), index finger ($p<0.05$), ring finger ($p<0.05$), and little finger ($p<0.05$), but not on middle finger. The SABD loads had a greater impact on thumb and index finger pressure, which increased by 35% and 28%, respectively. For the other three fingers, the impact of SABD was less pronounced (11% for middle finger, 22% for ring finger, 17% for little finger); 2) finger flexion pressure was generated during a hand open attempt in individuals with severe stroke. However, the age-matched able-bodied group did not generate finger flexion pressure during a hand open attempt. Future work will look at the effect of SABD in moderately impaired individuals with stroke to find out what levels of SABD will result in the inability to open the paretic hand.

Disclosures: Y. Lan: None. **J. Yao:** None. **J. Dewald:** None.

Nanosymposium

771. Stroke Recovery

Location: 152B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 771.09

Topic: C.21.Stroke Recovery

Support: NIH NINDS R21 NS082894

Title: Optogenetic stimulation of cerebellar dentate nucleus promotes persistent recovery after stroke

Authors: *M. Y. CHENG, A. M. SHAH, E. H. WANG, A. R. BAUTISTA, G. SUN, G. K. STEINBERG;
Neurosurg., Stanford Univ., Stanford, CA

Abstract: Functional recovery after stroke has been observed in both human and animal studies. After injury, the brain undergoes activity-dependent reorganization and rewiring, and this can occur in perilesional or distant brain regions. Post-stroke brain stimulations are promising

neurorestorative techniques as they allow direct manipulation of the target area's excitability. Previously we have demonstrated that optogenetic neuronal stimulation of the ipsilesional primary motor cortex promotes functional recovery. To determine an optimal brain stimulation target, we test whether optogenetic neuronal stimulation of the contralesional cerebellar dentate nucleus (cLCN) can promote recovery. We hypothesize that stimulation of cLCN may be more effective, as it sends excitatory outputs to multiple motor and premotor areas. Stroke mice were stimulated from day5-14 post-stroke and sensory-motor behavior test was used to evaluate recovery. Our data showed that stimulated mice recovered quickly, with significant improvement in distance traveled as early as day 7, and faster speed at day14 post-stroke. To evaluate whether the effect of cLCN stimulation was persistent, we tested the effects of short stim (day5-14) and long stim (day5-28) on recovery. Interestingly, the short stim group continued to recover after day14 without further stimulations and the long stim group did not further enhance recovery, indicating that functional outcome of cLCN stimulation is persistent, and prolonged stimulations may not be necessary to achieve permanent recovery. Analysis of pCREB activation showed that cLCN stimulation activates the dentatothalamocortical pathway, including LCN, thalamus, premotor and motor areas. Current studies examine the mechanisms of cLCN-induced recovery, including synaptic and plasticity markers. Our study highlights cLCN as a promising brain stimulation target for stroke recovery.

Disclosures: **M.Y. Cheng:** None. **A.M. Shah:** None. **E.H. Wang:** None. **A.R. Bautista:** None. **G. Sun:** None. **G.K. Steinberg:** None.

Nanosymposium

771. Stroke Recovery

Location: 152B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 771.10

Topic: C.21.Stroke Recovery

Title: High-cost training to recondition movement variability

Authors: *A. K. SHAH[1], J. L. PATTON[2,3], I. SHARP[2], E. LAZZARO[3];
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Abstract: Movement variability can be a problem in human performance, especially in the recovery following stroke or other neurotrauma, because it can cause people to exceed task limits and cause danger or discomfort to the individual, such as falls or accidents in the workplace or

other events of “high cost”. “Stiff” obstacles have been shown to reshape movement paths because they may be modeled by the nervous system as *boundaries* to avoid (Chib et al, 2004, J. Neurophysiol 95 (2) 1068-1077). Here we explore another such haptic method to reduce and reshape variability. Using a 3D reaching interception task, we investigated the potential to reshape movement variability within a predefined “safe” region by imposing forces of constant magnitude when the subject was out of the region. We randomly assigned 18 healthy and 10 stroke subjects into Control and treatment groups. All subjects had to attempt 600 interceptions of a projectile traveling at them. The haptic treatment groups experienced “limit-push” forces, which provided thrust away from the workspace center if the subject moved out of an invisible “safe” box-shaped region (during the treatment phase, trials 201-400 of the 600). We hypothesized that treatment groups would learn to avoid the boundaries of the safe region, with effects that lasted beyond the treatment phase. We evaluated nearest distance-to-edge, which increased if subjects remained further from the edge of the boundary. Results revealed that by the end of the treatment phase, the nearest distance-to-edge for the healthy group was 5.0 ± 2.6 cm (average ± standard deviation) higher than the healthy controls (t-test, $p<0.01$). Similarly, the stroke treatment group’s nearest distance-to-edge was 8.7 ± 7.8 cm higher than stroke control ($p<0.033$). We conclude that high-cost learning such as limit push may offer a novel paradigm for reshaping movement variability in both healthy human performance training and neurorehabilitation.

Disclosures: **A.K. Shah:** None. **J.L. Patton:** None. **I. Sharp:** A. Employment/Salary (full or part-time):; Televisa Internacional. **E. Lazzaro:** None.

Nanosymposium

771. Stroke Recovery

Location: 152B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 771.11

Topic: C.21.Stroke Recovery

Support: Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (A085136)

the Korea Government Ministry of Education, Science and Technology under Grant (2009-0069165)

the Korea Research Foundation Grant funded by the Korean Government (KRF-2007-331-E00126)

Title: Effects of mesenchymal stem cell treatment on the expression of matrix metalloproteinase and angiogenesis in ischemic stroke recovery

Authors: *H. NAM[1], I. KWON[2], B. LEE[1], O.-H. LEE[1], J. HEO[2];
[1]Dept. of Neurol., [2]Dept. of Neurology,, Yonsei Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: *Background and Purpose;* Earlier treatment with mesenchymal stem cell (MSC) could produce a greater effect on stroke recovery since the pathophysiological targets of ischemic brain dramatically change according to disease course. However, few studies investigated the effects of MSC transplantation in hyperacute period. MSC treatment can induce angiogenesis. Therapeutic stimulation of angiogenesis is important in recovery phase after ischemic stroke. On the contrary to the harmful role of Matrix metalloproteinases (MMP) in ischemic stroke, evidences suggested that MMP-2 is a key player in vascular development and angiogenesis. Because MMP modulate extracellular matrix, pro-anigogenic property of MSC treatment may be mediated by MMPs. We sought the optimal time window of MSC transplantation and investigated the MSC-induced MMP-2 elevation contributed to angiongensis in ischemic stroke recovery. *Methods;* Male Sprague-Dawley (SD) rats were subjected to permanent middle cerebral artery occlusion (MCAO). Human bone marrow-derived MSC (2×10^6) were transplanted after MCAO. We firstly sought the optimal time point of MSC administration after MCAO. MSCs were injected intravenously at 1 h, 1 day, or 3 days after MCAO. Secondarly, we investigated whether MSC-induced MMP elevation contributes to angiongensis. Gelatin zymography for MMP-2 and 9 was performed at 1, 4, 7, 10, and 14 days after MCAO. Neurological dificits were assessed using the rotarod, Longa score, and modified Neurologic Severity Score before and after MCAO. Infarction volume was measured with triphenyltetrazolium chloride staining. Vascular density at 10, and 14 days after MCAO was ascertained by collagen type 4 immunostaining. Immunofluorescence stainings for MMP-2, NeuN, OX-42, GFAP and collagen type 4 were also conducted. *Results;* Among the MSC transplanted rats, the MSC 1-hour group showed better recovery in the rotarod test ($P = 0.023$) and Longa score ($P = 0.018$) than saline injection group. MMP-2 activity was higher in MSC 1-hour group ($P = 0.028$). The MSC 1-hour group had smaller infarction volume. Collagen type 4 immunohistochemistry revealed that higher vascular density in MSC 1-hour group. Expression of MMP-2 after MSC transplantation was not colocalized with neuron (NeuN) or microglia (OX-42), but astrocyte (GFAP) and type 4 collagen in ischemic boundary area. *Conclusions;* We found that 1-hour MSC transplantation induced higher MMP-2 activity, better neurological improvement, smaller infarction volume, and higher vascular density. It could be speculate that MSC-induced MMP-2 elevation may be one of key mechanisms in angiogenesis and brain remodeling after ischemic stroke.

Disclosures: **H. Nam:** None. **I. Kwon:** None. **B. Lee:** None. **O. Lee:** None. **J. Heo:** None.

Nanosymposium

771. Stroke Recovery

Location: 152B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 771.12

Topic: C.21.Stroke Recovery

Support: NIH R03 HD073566

Title: Pairing forced aerobic exercise with task practice to augment the recovery of motor function following stroke: A randomized clinical trial

Authors: *S. LINDER[1], A. ROSENFELDT[2], A. PENKO[2], M. RASANOW[2], C. CLARK[2], J. L. ALBERTS[2];
[1]Cleveland Clin., Olmsted Falls, OH; [2]Cleveland Clin., Cleveland, OH

Abstract: OBJECTIVE: To determine the effects of forced aerobic exercise (FE) on the recovery of motor and non-motor function in individuals with hemiparesis due to stroke. BACKGROUND: While the cardiovascular benefits of aerobic exercise training is well documented in individuals with stroke, the potential role of aerobic exercise as it relates to neuroplasticity and the recovery of motor function has not been systematically investigated. In our previous work in individuals with PD, we developed a FE paradigm on a stationary bicycle to augment the voluntary efforts of patients, allowing them to achieve and maintain a rate of exercise thought to elicit neurophysiological changes in the brain resulting in improvements in motor and non-motor functioning, in addition to increased cortical activity and functional connectivity on neuroimaging. We have implemented a similar training protocol in individuals with stroke, pairing one of two forms of aerobic exercise (forced or voluntary-rate) in close temporal proximity with upper extremity repetitive task practice (RTP) to augment motor recovery. We hypothesized that intensive aerobic exercise training will serve to prime the central nervous system and enhance the recovery of motor function when paired with RTP to a greater degree than time-matched RTP alone. DESIGN/METHODS: Individuals 6-12 months following stroke were enrolled in this rater-blind, randomized clinical trial. Participants were randomized to one of the following time-matched intervention groups: 1) FE coupled with RTP; 2) Voluntary-rate aerobic exercise coupled with RTP; or 3) RTP only. Outcome measures included the Wolf Motor Function Test (WMFT), Fugl-Meyer Assessment (FMA), Six-minute walk test, Centers for Epidemiologic Studies Depression Scale (CES-D), and Stroke Impact Scale (SIS). RESULTS: Preliminary findings reveal improvements in WMFT, FMA, SIS, and CES-D scores in all three intervention groups (despite both aerobic exercise groups receiving 44% less RTP time and repetitions), with trends favoring the FE+RTP group in motor function outcomes and

depression. Improvements in VO2peak in both aerobic exercise training groups correlated with exercise compliance, defined as percent time spent within target heart rate zone. The RTP group did not demonstrate improvements in VO2peak. CONCLUSIONS: Individuals with chronic stroke can safely participate in intensive aerobic exercise. FE is a novel and efficacious intervention that can improve cardiovascular health and may augment the recovery of UE motor function post-stroke.

Disclosures: S. Linder: None. **A. Rosenfeldt:** None. **A. Penko:** None. **M. Rasanow:** None. **C. Clark:** None. **J.L. Alberts:** None.

Nanosymposium

771. Stroke Recovery

Location: 152B

Time: Wednesday, November 19, 2014, 1:00 PM - 4:15 PM

Presentation Number: 771.13

Topic: B.04. Ion Channels

Support: CoPOC Inserm

Fondation pour l'innovation thérapeutique Béatrice DENYS

ANR- 12-EMMA-0036

Title: Is optimized tissue plasminogen activator (optPA) the future of intracerebral haemorrhage treatment?

Authors: *J. PARCQ, R. GOULAY, D. VIVIEN;
GIP Cyceron - INSERM, Caen, France

Abstract: Intracerebral haemorrhage (ICH) is a life-threatening disease that results from a massive extravasation of blood anywhere within the brain parenchyma. Up to now, no treatment has been approved by the authorities for ICH. Of the studies completed so far, none have shown a clear beneficial result. A promising treatment is now being evaluated: a combination of minimally invasive surgery and clot lysis with recombinant tissue Plasminogen Activator (tPA, actilyse®) to remove ICH (MISTIE clinical trial). Preliminary results from the MISTIE-II trial have shown that blood clots in the brain can be removed quickly, safely and successfully by tPA-induced fibrinolysis. tPA is a powerful fibrinolytic but nevertheless putative side effects on cells of the neurovascular unit, especially neurons are highlighted. Indeed tPA has been reported to promote noxious effects including neurotoxicity mediated by over-activation of NMDA

receptors. In a model of ICH in pigs tPA treatment has been shown to double the volume of residual edema, thus reducing benefits of hematoma drainage. In the same model, MK801 an antogonist of NMDAR and PAI-1 a tPA-inhibitor, were both capable to reduce tPA-dependent neurotoxicity. On the basis of over 10 years of research investigating the molecular mechanisms by which tPA mediates neurotoxicity, we have carried out an ambitious program to create a brain-safe thrombolytic. We are now poised to propose an original de-poisoned thrombolytic derived from the current human tPA, named Optimized tPA (OptPA). OptPA is a Human tissue plasminogen activator containing 2 point mutations. It is produced in HEK cells and purified thanks to a 6xHis tag. OptPA is a fibrinolytic as well as tPA, assessed using different methods from human plasma-derived euglobulin clot lysis time, human plasma clot lysis time and rotation thromboelastometry with human blood samples. Its competitive advantages over tPA is to lack NMDAR-dependent neurotoxicity in vitro (in a model of mouse primary culture of cortical neurons) and in vivo (models of NMDA-dependent excitotoxicity in rodents). We thus propose OptPA as the future for improved fibrinolysis following ICH in humans.

Disclosures: J. Parcq: None. **R. Goulay:** None. **D. Vivien:** None.

Nanosymposium

772. Functional Organization of the Human Visual Cortex

Location: 144A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 772.01

Topic: D.04. Vision

Support: NWO Grant 406-11-197

NWO Grant 451-09-030

NWO Grant 452-08-008

Title: The cortical representation of binocular stimuli across human visual areas

Authors: *M. BARENDREGT[1,2], B. ROKERS[2,1], S. O. DUMOULIN[1];
[1]Utrecht Univ., Utrecht, Netherlands; [2]Univ. of Wisconsin-Madison, Madison, WI

Abstract: A single object produces two retinal images and the horizontal offsets between the retinal images form the basis of stereoscopic depth perception. The canonical view of visual processing is that visual information goes through a number of transformations, but that the retinotopic representation is essentially maintained at each stage. This implies that two retinal

projections of the object are maintained as opposed to the single (cyclopean) percept of the object. Here we provide evidence that the transformation from V1 to V3 involves a transformation of a retinotopic to a cyclopean representation of the visual scene. Using high-field fMRI (7T), we measured BOLD responses in early visual cortex to a stimulus containing binocular disparity. The stimulus consisted of a bar-shaped aperture that revealed a 1/f pink noise pattern with a small horizontal offset between the two eyes. In two control experiments, we also measured BOLD responses to (1) the same stimulus, but with temporal interleaving of left and right eye images, and (2) the same stimulus but without the horizontal offset. The experimental stimulus results in the percept of a single object located in depth, whereas the control stimuli produce the percept of (1) two alternating objects or (2) one object located in the fixation plane. Next, we estimated population receptive field (pRF) properties and identified whether the cortical representation reflects that of a single object image or two retinal images. In the control experiments, we found that the cortical representation of the stimuli in V1 to LO2 corresponded to two offset retinal images, and a single object image respectively. This result reveals the sensitivity of our method to identify whether a cortical representation reflects a unified or two retinal images. Next, we applied the same procedure to the stimulus containing binocular disparity. In V1, we found a cortical representation consistent with two retinal images, suggesting that the horizontal offset from the retinas is still present in the cortical representation of the stimulus. In the V2 visual field map the results were indeterminate. From visual field map V3 onwards (V3A, LO1, LO2), we find a single image cortical representation analogous to the single object that produced the two retinal images. In summary, we are able to dissociate cortical representations based on a single or two offset retinal images. Furthermore, the V1 cortical representation corresponds to the location of the stimulus on each retina. In later visual areas (V3 and onward) however, the cortical representation is not related to the retinal location of the stimulus, but follows a cyclopean representation of the visual scene.

Disclosures: **M. Barendregt:** None. **B. Rokers:** None. **S.O. Dumoulin:** None.

Nanosymposium

772. Functional Organization of the Human Visual Cortex

Location: 144A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 772.02

Topic: D.04. Vision

Support: NSF: BCS 0920865

NIH: NRSA 1F32EY018533-01

Title: Smaller population receptive fields in extra striate and face selective regions of developmental prosopagnosics

Authors: *N. WITTHOFT[1], S. POLTORATSKI[2], M. NGUYEN[3], G. GOLARAI[1], A. LIBERMAN[4], K. F. LAROCQUE[1], M. E. SMITH[5], K. GRILL-SPECTOR[1];
[1]Psychology, Stanford Univ., Stanford, CA; [2]Psychology, Vanderbilt, Nashville, TN; [3]Psychology, New York Univ., New York, NY; [4]Helen Willis Neurosci. Inst., Univ. of California, Berkeley, Berkeley, CA; [5]Psychology, UCSD, San Diego, CA

Abstract: Congenital/developmental prosopagnosics (DPs) show a pronounced deficit in recognizing other people but not other obvious visual problems. This impairment is thought to be heritable and reflect genetic differences and differential development as a result. Empirical results suggest that normal face recognition is dependent on holistic processing, and that this type of processing may be at least partially impaired in DP. While there is some debate over the meaning of the term holistic, this type of processing certainly depends on the integration of features over a significant portion of the visual field, suggesting the hypothesis that DPs may show deficits in neural spatial integration, particularly in visual regions processing faces. We tested this hypothesis by collecting data from retinotopy and category localizer experiments using fMRI in 7 developmental prosopagnosics and 14 controls. We used this data to define face selective regions as well as visual field maps in all subjects. Retinotopy data were analyzed using a population receptive field (pRF) model, which allowed us to define the location and spatial extent of pRFs in retinotopically modulated voxels, including those in face selective regions. We found that pRFs and visual field coverage in V1-V3 are similar across DPs and controls. However, higher-level regions show a prominent lack of large receptive fields in DPs compared to controls, although the extent of visual field coverage is similar for the anterior visual field maps across groups. This difference is particularly pronounced in face selective regions on the inferior occipital and lateral fusiform gyri. In controls, voxels in face-selective regions show fairly large and foveally located pRFs extending to the ipsilateral visual field. In DPs, pRF sizes are greatly reduced though still centered foveally. As a result DPs have a substantially limited visual field coverage and lesser ipsilateral visual field coverage in face-selective regions. These findings are broadly consistent with the notion that DPs may rely on feature based strategies when processing faces. However, given the widespread nature of differences outside of early visual cortex, there may be other subtle differences in their visual performance relative to controls.

Disclosures: **N. Witthoft:** None. **K.F. Larocque:** None. **S. Poltoratski:** None. **M. Nguyen:** None. **A. Liberman:** None. **G. Golarai:** None. **M.E. Smith:** None. **K. Grill-Spector:** None.

Nanosymposium

772. Functional Organization of the Human Visual Cortex

Location: 144A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 772.03

Topic: D.04. Vision

Support: NWO VIDI Grant 13339

ERC Advanced iCONNECT

Title: Functional mri and electrocorticography correspondence in human extra-striate cortex (hmt+)

Authors: A. GAGLIANESE[1], M. VANSTEENSEL[1], B. HARVEY[2], S. O. DUMOULIN[2], N. F. RAMSEY[1], *N. PETRIDOU[1];
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Abstract: Recent work has shown that spectral changes in high frequency broadband (HFB) power recorded by intra-cranial electrocorticography (ECoG) reflect neuronal population spiking activity and are directly correlated with BOLD responses [1]. Moreover, it has been shown that HFB power can predict the non-linear BOLD saturation in sensorimotor system, in the case of increasing frequency of movement [2]. Here, we extend this observation to the human Middle Temporal complex (hMT+), a higher order brain area involved in visual motion processing [3]. We investigate the coupling between BOLD fMRI responses and HFB (65-95 Hz) ECoG signals using a visual stimulus moving at different speeds. Our subject was a patient who underwent pre-surgical 3T fMRI and subsequent implantation of an ECoG array for the purpose of epilepsy monitoring (128 channel recording, 512 Hz sampling rate). During both measurements, the subject was presented with a visual stimulus that consisted of a high-contrast black-and-white dartboard with spatial frequency of 3deg/cycle. The stimulus expanded from fixation point at two temporal frequencies (1Hz and 5Hz in two separate runs). We functionally localized the hMT+ complex by computing BOLD responses for each voxel in the brain using a deconvolution approach ($p<0.05$ corrected), and we extracted HFB power from ECoG recordings at the same location ($p<0.05$ corrected). To investigate the relationship between neuronal activity and vascular responses, we compared measured BOLD responses with BOLD responses predicted from: 1) the stimulus time course, e.g. temporal frequency changes, 2) the HFB power measured by ECoG. We found consistent and specific HFB power augmentation in response to the two different temporal frequencies of the visual motion stimuli. With low temporal frequency stimulation, both the stimulus time course and HFB power predicted BOLD responses well.

However, with high temporal frequency stimulation the HFB-based prediction of BOLD responses was more accurate. This demonstrates that BOLD activity under the electrode could be better predicted by HFB power than by the stimulus driving this neuronal activity. This suggests a close match between neuronal population activity and BOLD responses. Moreover, our finding suggests that neuronal population activity as reflected by HFB power may predict the non-linear BOLD saturation in the case of visual motion perception, as demonstrated in the sensorimotor system [2]. [1] Hermes, D. et al. (2012), Human Brain Mapping 33:1689-1699 [2] Siero, J. et al. (2013), JCBFM 33, 1448-1456 [3] Amano, K. et al. (2009), Journal of Neurophysiology, vol. 102, pp. 2704 -2718.

Disclosures: **A. Gaglianese:** None. **M. Vansteensel:** None. **B. Harvey:** None. **S.O. Dumoulin:** None. **N.F. Ramsey:** None. **N. Petridou:** None.

Nanosymposium

772. Functional Organization of the Human Visual Cortex

Location: 144A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 772.04

Topic: D.04. Vision

Support: NWO Vidi grant 13339 (N.P.)

Vidi grant 452-08-008 (S.O.D.)

Title: Laminar profiles of population receptive field (pRF) size from primary visual cortex

Authors: *A. FRACASSO[1], N. PETRIDOU[2], S. DUMOULIN[1];
[1]Utrecht Univ., Utrecht, Netherlands; [2]Univ. Med. Ctr. (UMC), Utrecht, Netherlands

Abstract: Introduction: Visual input from the eyes arrives predominantly in granular layers of primary visual cortex (V1), then rapidly spreads across lamina and through horizontal connections before reaching extra-striate cortex. In line with this laminar hierarchy, neurophysiology measurements show that neurons in granular layers have small visual receptive fields (RF's), which increase in supra- and infra-granular layers (Hubel & Wiesel, 1968). This finding has been replicated across different species and modalities suggesting a general organization principle of primary sensory cortex (Chapin, 1986). Here, we provide human in vivo evidence of population RF (pRF) size and surrounds across lamina. Methods: We acquired sub-millimeter (0.7mm, isotropic, TR=4s) ultra-high field functional MRI (7T) while participants

(n=3) viewed an expanding and contracting contrast-defined ring centered on the fovea (4 deg radius). We estimated eccentricity, size and surround of the pRF for each voxel in V1, using a forward model (Dumoulin & Wandell, 2008). To avoid small realignment related errors, laminar distance map was based upon a gray matter/white matter manual segmentation derived from a combination of the mean image of reconstructed fMRI amplitude and unwrapped phase (equivalent to anatomical T2*weighted images, Duyn et al, 2007). pRF size by eccentricity relation was estimated in 10 points across laminar distance. For each point, the corresponding pRF size was obtained. Results: pRF size increased as a function of eccentricity. Across lamina, pRF sizes and surrounds are smaller in granular compared to supra- and infra-granular layers. pRF size/ surround ratio is approximately constant across lamina. Conversely, signal amplitude increases monotonically towards the pial surface, as can be expected from the known laminar vascular distribution (Duvernoy et al, 1981, Polimeni et al, 2010). Thus these pRF size and surround variations cannot be explained by vascular differences across layers. Results are consistent across participants and the same participant on different days. Conclusions: We reconstructed pRF size and surround across lamina in humans using in vivo sub-millimeter fMRI at ultra-high field. In line with neurophysiology, we reveal a laminar hierarchy in pRF size and surrounds, being smaller in granular layers and gradually increasing towards infra and supra granular layers. These results extend the systematic pRF size variation across the visual field map hierarchy to laminar hierarchy within a visual field map.

Disclosures: **A. Fracasso:** None. **N. Petridou:** None. **S. Dumoulin:** None.

Nanosymposium

772. Functional Organization of the Human Visual Cortex

Location: 144A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 772.05

Topic: D.04. Vision

Support: WARF grant

Title: Altered white matter integrity in visual pathways as a result of amblyopia

Authors: ***B. ALLEN**[1], D. SPIEGEL[2], B. THOMPSON[2], F. PESTILLI[3], B. ROKERS[1]; [1]Psychology, Univ. of Wisconsin - Madison, Madison, WI; [2]Optometry and Vision Sci., Univ. of Auckland, Auckland, New Zealand; [3]Psychology, Stanford Univ., Stanford, CA

Abstract: Introduction Complete blindness in one eye is associated with reduced lateral geniculate nucleus (LGN) volume (Kupfer & Plamer, 1964) and reduced density (Fifkova, 1970) as well as altered diffusivity of the optic radiations (Levin et al., 2009). The effect of amblyopia, which involves intracortical suppression rather than absence of visual input, is less established, although at least one study suggests similar underdevelopments of optic radiation in amblyopes (Ming-xia et al., 2007). In this study, we are interested in the effect of amblyopia on the white matter integrity of both thalamic and cortical projections into extrastriate area MT+ (which is involved both in the processing of visual motion and depth) since amblyopia results in degraded or absent binocular depth perception, while seemingly sparing motion integration. Methods We used FreeSurfer (Desikan et al., 2006; Fischl et al., 2008) to segment and identify human MT+ and V1 in 10 amblyopic and 10 normally sighted participants. The pulvinar (PLN) and LGN of the thalamus were identified based on anatomical landmarks. We obtained diffusion-weighted MRI images (2 mm isotropic resolution; 32 diffusion directions; b0 = 1000). Constrained spherical deconvolution and probabilistic tractography (Lmax=6; Tournier et al., 2012), which are robust against mischaracterization of pathways resulting from crossing fibers, were used to identify fiber tracts. We generated a large set of candidate tracts (500,000/pathway) between LGN, PLN, V1, and MT+ for each participant. Pathway estimates were then sampled at 100 evenly spaced nodes and the middle 60 nodes for each pathway were used in the final analyses. The data were averaged between hemispheres but separated by group (amblyopes and controls). Results Amblyopes exhibited significantly reduced white matter integrity in in regions comprising the optic radiation, the thalomo-extrastriate (LGN to MT+ and PLN to MT+) pathways. Discussion Our results suggest that amblyopia affect the white matter integrity of both striate- and extrastriate-projections from thalamic nuclei. These results indicate that amblyopes provide a valuable clinical population to study the contribution of thalomo-striate and poorly understood direct thalomo-extrastriate projections, and can help elucidate the contribution of these pathways to visual motion and depth perception.

Disclosures: B. Allen: None. **D. Spiegel:** None. **B. Thompson:** None. **F. Pestilli:** None. **B. Rokers:** None.

Nanosymposium

772. Functional Organization of the Human Visual Cortex

Location: 144A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 772.06

Topic: D.04. Vision

Support: NSF Grant DGE-114747

NIH Grant EY02231801A1

Title: Functionally defined white matter reveals segregated pathways in human ventral temporal cortex associated with category-specific processing

Authors: *J. GOMEZ[1,2], F. PESTILLI[3], N. WITTHOFT[3], G. GOLARAI[3], A. LIBERMAN[4], S. POLTORATSKI[5], J. YOON[6], K. GRILL-SPECTOR[3];
[2]Neurosciences Dept., [3]Psychology Dept., [1]Stanford Univ., Stanford, CA; [4]Helen Wills Neurosci. Inst., Univ. of California, Berkeley, CA; [5]Psychology Dept., Vanderbilt Univ., Nashville, TN; [6]Psychology Dept., New York Univ., New York, NY

Abstract: A fundamental yet unanswered question in neuroscience is whether or not white matter properties associated with specific visual networks selectively contribute to category-specific processing. In a novel protocol we combined measurements of behavior, functional selectivity of human ventral temporal cortex (VTC), and advanced diffusion-weighted imaging (DWI) of white matter structure in the same subjects (n=9 typical adults). Extracting fiber tracts that pass near a face-selective region on the middle fusiform gyrus (mFus-faces) or a place-selective region on the collateral sulcus (CoS-places), we find two parallel white matter pathways along the ventral temporal lobe connecting to either face- or place-selective cortex. Diffusion properties of portions of these tracts adjacent to face- and place-selective regions of VTC correlate with behavioral performance for face or place processing, respectively. Strikingly, in adults with developmental prosopagnosia (face blindness; n=7), this correlation is inverted near face-selective cortex, but not near place-selective cortex. This suggests that an atypical structural-functional relationship near face-selective cortex may have behavioral consequences. These results demonstrate that white matter connectivity differences associated with functional divisions in high-level visual cortex are behaviorally relevant. Such segregated pathways offer evidence for parallel processing streams in human VTC, mirroring axonal tracing studies in macaques. We argue that examining the interplay between cortical function, anatomical connectivity, and visual behavior is integral to understanding functional networks and their role in producing visual abilities and deficits.

Disclosures: J. Gomez: None. **F. Pestilli:** None. **N. Witthoft:** None. **G. Golarai:** None. **A. Liberman:** None. **S. Poltoratski:** None. **J. Yoon:** None. **K. Grill-Spector:** None.

Nanosymposium

772. Functional Organization of the Human Visual Cortex

Location: 144A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 772.07

Topic: D.04. Vision

Support: NEI RO1 Grant EY019272

NEI RO1 Grant EY024019

DoD W81XWH-08-2-0146

DFG

Plasticise consortium HEALTH-F2-2009- 223524

Max Planck Society

Title: Organization of human area V5/MT+ and sensitivity to motion coherence after lesions of the primary visual cortex

Authors: *A. PAPANIKOLAOU[1], G. A. KELIRIS[1], S. LEE[2], T. D. PAPAGEORGIOU[2], U. SCHIEFER[3], N. K. LOGOTHETIS[1], S. M. SMIRNAKIS[2];
[1]Max-Planck Inst. For Biol. Cybernetics, Tuebingen, Germany; [2]Baylor Col. of Med., Houston, TX; [3]Ctr. for Ophthalmology, Univ. of Tuebingen, Tuebingen, Germany

Abstract: Partial loss of the primary visual cortex (V1) and/or its inputs leads to a scotoma of the contralateral visual hemifield, the extent of which corresponds retinotopically to the region affected. However, some patients have been found to retain a small amount of residual visual sensitivity within the blind field, a phenomenon termed blindsight, suggesting the existence of alternate pathways that transmit information from the retina to cortex effectively bypassing V1. Blindsight has been associated with activity observed in the middle temporal area complex (V5/MT+) following V1 lesions. An important issue is how the properties of area hV5/MT+, like retinotopic organization and sensitivity to motion, change following V1 lesions. We measured responses in human area V5/MT+ in 5 patients with homonymous visual field defects as a result of area V1 or optic radiation lesions using functional magnetic resonance imaging (fMRI). First, we investigated whether the organization of area hV5/MT+ changes following V1 damage. To do so, we used a recent method that estimates population receptive field (pRF) topography in the visual cortex (Lee et al., A new method for estimating population receptive field topography in visual cortex, NeuroImage, 2013). FMRI measurements were obtained during the presentation of a moving bar stimulus while the subjects were fixating. The pRF topography of area hV5/MT+ was compared with that of control subjects stimulated with matching “artificial scotomas”. In addition, we measured the sensitivity of area hV5/MT+ to coherent motion using random dot kinematograms (RDK) for both patients and controls. RDK patches were presented either inside the visual field scotoma or in the contralateral healthy part of the visual field. Subjects were

instructed to report the direction of motion of the presented RDK while fixating. In both cases we found responses in hV5/MT+ arising inside the scotoma, independent of area V1 input, suggesting the existence of a functional alternate pathway bypassing area V1. The retinotopic organization of hV5/MT+ differed between patients and controls under the artificial scotoma condition, suggesting a degree of reorganization. The blood oxygen level-dependent (BOLD) response of area hV5/MT+ to RDK coherent motion stimuli also differed between patients and controls, and was dependent on which side was attended. Studying how the properties of visual areas change after injury may allow us to design better rehabilitative strategies in the future.

Disclosures: A. Papanikolaou: None. **G.A. Keliris:** None. **S. Lee:** None. **T.D. Papageorgiou:** None. **U. Schiefer:** None. **N.K. Logothetis:** None. **S.M. Smirnakis:** None.

Nanosymposium

772. Functional Organization of the Human Visual Cortex

Location: 144A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 772.08

Topic: D.04. Vision

Support: NIH Grant EY10217

Title: Vascular supply of the cerebral cortex is specialized for cell layers but not columns

Authors: *J. C. HORTON[1], J. R. ECONOMIDES[2], D. L. ADAMS[2];
[1]Beckman Vision Ctr., Univ. California, SAN FRANCISCO, CA; [2]UCSF, San Francisco, CA

Abstract: Neurons in the primate cerebral cortex are organized into horizontal layers and vertical columns. Layers are associated with obvious variations in cell density and type, whereas columns are defined by common receptive field properties. The vascular supply to layers and columns was compared in macaque primary visual cortex by labeling red blood cells via their endogenous peroxidase activity in unperfused tissue. Alternate sections were processed for cytochrome oxidase to reveal the “patches” or “blobs”, which anchor the interdigitated column systems of striate cortex. As established previously, more densely populated cell layers received the most profuse blood supply. In the superficial layers the blood supply was organized into microvascular lobules, consisting of a central venule surrounded by arterioles. The identity of each vessel - venule or arteriole - was established by matching it with the point where it penetrated the cortex from a parent artery or vein in the pial circulation. Although microvascular lobules and cytochrome oxidase patches had a similar periodicity, they bore no mutual

relationship. The size and density of penetrating arterioles and venules did not differ between patches and interpatches. The overall amount of red blood cell label in patches and interpatches was equal. Moreover, patches and interpatches were supplied by an anastomotic pial arteriole system, with no segregation of blood supply to the two compartments. Often a focal constriction was located at the origin of pial arterial branches, suggesting the presence of vascular sphincters. Our data refute the concept that the vascular bed of the cortex is plumbed preferentially to serve columns. There was no difference in the system of blood vessels that supplies CO interpatches and patches, despite the fact that patches have greater metabolic activity. Local control of cortical perfusion may be regulated by vascular sphincters that match blood flow to shifts in metabolic demand.

Disclosures: J.C. Horton: None. **J.R. Economides:** None. **D.L. Adams:** None.

Nanosymposium

772. Functional Organization of the Human Visual Cortex

Location: 144A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 772.09

Topic: D.04. Vision

Support: EY-12925

EY-014645

EC FP-7 Marie Curie IOF fellowship no. 272834

Title: Estimating cortical reorganization and filling-in using fMRI population receptive field (pRF) mapping

Authors: *I. FINE[1], J. M. THOMAS[1], P. BINDA[3], E. B. RUNESON[2], G. M. BOYNTON[1]; [1]Dept. of Psychology, [2]Univ. of Washington, Seattle, WA; [3]Dept. of Translational Res. on New Technologies in Med. and Surgery, Univ. of Pisa, Pisa, Italy

Abstract: Purpose: Unbiased retinotopic map estimates in early visual areas can be obtained near the boundaries of scotoma if the pRF fitting algorithm takes the scotoma into account and a randomized "multifocal" stimulus sequence is used (Binda et al., 2013). However pRFs estimated with a spatiotemporally predictable drifting bars sequence are less biased than predicted, and are therefore over-corrected when the scotoma is taken into account. One possible explanation for this is ‘neural filling-in’. Being able to separately quantify the effects of cortical

reorganization and ‘filling-in’ within scotoma is important, since differences in filling-in have been noted in subjects with vision loss (Masuda et al. 2008). Here we show that the differences in the response to the multifocal and drifting bar stimulus can be used to visualize ‘filling in’ within a simulated scotoma. Methods: In 3 subjects we measured BOLD responses to drifting bars drifting and a multifocal (spatio-temporally unpredictable) stimulus. This was done with and without a mask occluding the central 2 degrees of the visual field, simulating a foveal scotoma. We found the pRFs that best predicted each voxel’s response over time using the full-field multifocal stimulus, across areas V1-V3. For all four stimuli, a model neural image was generated for each time point by multiplying each voxel’s pRF Gaussian by the stimulus image (convolved with the hemodynamic response) at that point in time. The actual neural image was generated for each time point by multiplying each voxel’s pRF Gaussian by the stimulus-driven BOLD signal at that point in time. For both model and real neural images, we calculated the mean intensity of the neural image in a central ROI (positioned in the middle of the scotoma) at each point in time. Results: For stimuli without a scotoma, the mean intensity over time of model and real neural images within the ROI were highly correlated. The multifocal stimulus with a scotoma was strongly correlated with the model neural image generated using the multifocal stimulus with a scotoma, suggesting a lack of filling-in. In contrast, the neural image obtained for the drifting bar stimulus with a scotoma was better correlated with the model neural image generated using the drifting bar stimulus without a scotoma - demonstrating significant neural filling in. Thus, our methods provide a way to separately quantify cortical reorganization and filling-in under condition of vision loss.

Disclosures: I. Fine: None. **J.M. Thomas:** None. **P. Binda:** None. **E.B. Runeson:** None. **G.M. Boynton:** None.

Nanosymposium

772. Functional Organization of the Human Visual Cortex

Location: 144A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 772.10

Topic: D.04. Vision

Support: ARC Future fellowship (FT120100816)

Title: An unbiased perspective of human visual cortex reveals new into insights orientation decoding

Authors: *T. A. CARLSON;
Macquarie Univ., Sydney, Australia

Abstract: Multivoxel pattern analysis (MVPA), or "decoding", methods confer heightened sensitivity to fMRI research by identifying patterns of activity across voxels associated with stimuli or cognitive states, which are invisible to traditional univariate analyses. The decodable information these methods discover and make use of, however, is often hidden within complex patterns of activation, obscuring its source. This is exemplified by two influential studies showing the orientation of visual gratings can be decoded from brain activity in human visual cortex using fMRI (Haynes and Rees, 2005; Kamitani and Tong, 2005). While it is well known that human visual cortex represents orientation, these studies were conducted at a scanning resolution insufficient to resolve orientation columns - orientation information therefore should not have been accessible. Without a clear source to explain this remarkable result, two theories have emerged. The hyper acuity account posits that imperfect sampling of orientation columns within fMRI voxels results in small biases that MVPA can exploit to recover stimulus orientation. The biased map account alternatively argues that the capacity to decode orientation is derived from a disproportionate number of neurons representing radial orientations (i.e. the radial bias). Such an imbalance would create biases within voxels, which in turn could be used to recover stimulus orientation. Both accounts notably assume that biases within voxels are the source of decodable information. In the present study, we tested whether this assumption is necessary. Using a classic model of visual cortex, we show that stimulus orientation can be decoded from an unbiased representation and identify the stimulus edges as a source of the decodable activity. We further show that the spatial distribution of this edge related activity masquerade as radial bias, and thus can also account for the observed radial bias in human fMRI studies. Our implementation of this classic model thus sheds new light on how stimulus orientation can be decoded from human visual cortex with fMRI.

Disclosures: T.A. Carlson: None.

Nanosymposium

773. Neuroscience and Psychology of Motherhood

Location: 140A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 773.01

Topic: E.03. Behavioral Neuroendocrinology

Support: NSF (IOS) 0914386

Title: Mental and physical (MAP) training: A neurogenesis-inspired intervention enhances health in homeless traumatized young mothers in the community

Authors: *T. J. SHORS[1], R. OLSON[2], M. CHANG[1], E. SELBY[1], M. BATES[3], B. ALDERMAN[2];
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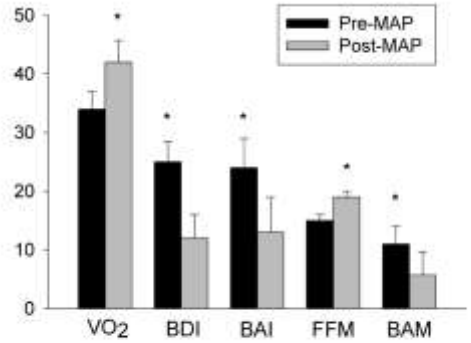
Abstract: Homelessness is one of the most stressful of life experiences, especially for a young mother with small children. Over the past year, we have been providing an intervention that was inspired from neurogenesis research in the laboratory (Shors 2014; Shors et al., 2014). Because the program combines mental and physical skill training, it is referred to as MAP Training (Curlik and Shors, 2012). In this study, we provided MAP Training to young mothers who were very recently homeless and suffering from traumatic life experiences including poverty, sexual assault and addictions. These young mothers reside at the Center for Great Expectations, a residential center located in Somerset, New Jersey. During each session, subjects engage in mental training with meditation (20-min sitting meditation and 10-min walking meditation) followed by physical skill training with 30-min of choreographed aerobic dance routines. MAP Training was provided twice a week for 8 weeks. Before and after MAP Training, subjects were tested for changes in psychological, neurophysiological and cardiac outcomes. After MAP training, oxygen consumption [VO2; measured in mL/kg*min)] in the mothers increased significantly ($p<0.001$). VO2 max is one of the most powerful predictors of physical health and longevity. The women were also less depressed (Beck's Depression Inventory; BDI), less anxious (Beck's Anxiety Inventory: BAI), more mindful (Five Faces to Mindfulness; FM), and engage in more loving interactions with their children (Being a Mother Scale; BAM). Overall, our data indicate that combining mental with physical skill training has a positive impact on the lives of young mothers and their children, thus helping break the cycle of poverty, abuse and addiction that often occurs in our most vulnerable



populations.

Disclosures: **T.J. Shors:** None. **R. Olson:** None. **M. Chang:** None. **E. Selby:** None. **M. Bates:** None. **B. Alderman:** None.

Nanosymposium

773. Neuroscience and Psychology of Motherhood

Location: 140A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 773.02

Topic: E.03. Behavioral Neuroendocrinology

Support: NSF Grant 0914386

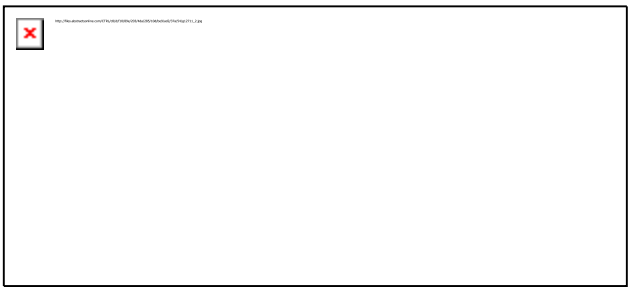
NIH- 5K12GM93854

Title: Sexual Conspecific Aggressive Response (SCAR): Sexual aggression directed at females in puberty disrupts maternal care for offspring

Authors: *K. E. TOBON[1,3], D. DURHAM[1], M. CHANG[1], A. MATTHEW[1], G. DIFEO[2], T. SHORS[2];
[2]Behavioral and Systems Nueroscience, Dept. of Psychology, [1]Rutgers Univ., Piscataway, NJ;
[3]Rutgers-Robert Wood Johnson Med. Sch., Piscataway, NJ

Abstract: Sexual aggression and assaults are especially stressful life experiences, especially for females in puberty. To examine the consequences of sexual aggression on maternal behavior, we developed an animal model of sexual aggression, termed Sexual Conspecific Aggressive Response (SCAR). During the procedure, a pubescent female rat is placed in contact with a sexually-aggressive male each day for 30 minute sessions. The males are very aggressive toward the females. During each experience, the male pinned the female ~10 times with nearly 30 ano-genital sniffs, significantly more than when placed with either adult rats of the opposite sex or same sex. The goal of this experiment was to determine whether exposure to the aggressive male during puberty would disrupt the development of maternal behaviors. It was hypothesized that pubescent virgin females that were exposed to the aggressive male during the SCAR procedure would require more time to learn to become maternal, as assessed by increased latency to develop maternal behaviors. During the SCAR procedure, pubescent females are then exposed to pups (PND1-10). The pups are continually replaced every 24 hours for 21 consecutive days, after daily SCAR exposures. The behaviors of the female toward the pups were recorded. Females that were not exposed to the adult male (No SCAR) developed maternal care and behaviors as evidenced by licking, retrieving, and sitting over the pups. The no-scar females express these

behaviors at 3-10 days of having pups in their homecage, whereas those exposed to the aggressive male (SCAR) females did not express maternal behaviors until 13-15 days later (p= 0.01). Whereas 100% of the no-scar group became maternally sensitized, only 33% of the SCAR females developed maternal behaviors within 17 days of having pups in their homecage constantly. These data suggest that the pubescent females that were repeatedly exposed to an aggressive adult male did not develop maternal behavior in a timely manner, if at all. Together, these data suggest that SCAR has deleterious consequences for the development of maternal behaviors.



Disclosures: **K.E. Tobon:** None. **D. Durham:** None. **M. Chang:** None. **A. Matthew:** None. **G. DiFeo:** None. **T. Shors:** None.

Nanosymposium

773. Neuroscience and Psychology of Motherhood

Location: 140A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 773.03

Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant 1F31MH099892

NIH Grant RO1HD057962

Title: Postpartum neuroplasticity in the midbrain serotonergic dorsal raphe: Potential mechanism for adaptations in maternal behavior and physiology

Authors: *M. A. HOLSCHBACH, J. S. LONSTEIN;
Neurosci. Program, Michigan State Univ., East Lansing, MI

Abstract: New mothers have experienced dramatic endocrine changes that elicit widespread neural plasticity underlying the similarly dramatic changes in their behavior and physiology. The

birth and development of new brain cells has been studied in several regions of the peripartum forebrain where reproductive state and maternal interactions with pups affect cytogenesis, but sites in the midbrain have never been reported. In these studies, we investigated cytogenesis in the dorsal raphe, the brain's main source of serotonin. Serotonin affects various aspects of postpartum socioemotional behaviors (caregiving, aggression, anxiety), as well as lactation, so neuroplasticity within the dorsal raphe could play a vital role for new mothers. Using bromodeoxyuridine (BrdU; 150 mg/kg) to label mitotic cells in virgin, pregnant, and postpartum rats, we recently discovered that cells born one week after parturition were less likely to survive than cells born during pregnancy. Next, we determined whether interaction with the litter might contribute to the postpartum reduction in cytogenesis. Using BrdU to label mitotic cells in postpartum females with or without their pups since parturition, we found that interacting with pups significantly reduced survival of new cells in the dorsal raphe. To determine if pup presence reduced cell survival by increasing the dams' circulating corticosterone levels, an ongoing experiment is assessing whether adrenalectomy increases cytogenesis in the dorsal raphe of dams with their pups. Lastly, to determine possible functional significance of this change in cytogenesis, we are currently examining serotonin content in the dorsal raphe across reproductive state. Research into neuroplasticity in the serotonin systems underlying the postpartum adaptations in socioemotional behaviors and physiology could help identify novel targets for ameliorating postpartum mental health disorders and facilitate interactions between mothers and their infants.

Disclosures: M.A. Holschbach: None. **J.S. Lonstein:** None.

Nanosymposium

773. Neuroscience and Psychology of Motherhood

Location: 140A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 773.04

Topic: E.03. Behavioral Neuroendocrinology

Support: NIMH grant R0084148

Title: Postpartum depressive-like behavior is accompanied by impaired maternal motivation and altered structural plasticity within the reward system

Authors: *A. HAIM[1], C. ALBIN-BROOKS[2], M. L. SHERER[2], E. MILLS[2], B. LEUNER[3]; [2]Psychology, [3]Psychology, Neurosci., [1]The Ohio State Univ., Columbus, OH

Abstract: Postpartum depression (PPD) is the most common complication following childbirth experienced by 20% of all new mothers. PPD is often accompanied by parenting deficits which neuroimaging studies suggest may be due to blunted responsiveness of brain regions involved in regulating reward and motivation, including the nucleus accumbens (NAc). Stress during pregnancy is major risk factor for PPD and in rodents gestational stress similarly induces depressive-like behavior during the postpartum period. Yet, the impact of gestational stress on the NAc of postpartum rodents remains unspecified. Here we examined whether increased postpartum depressive-like behavior following exposure to gestational stress would be accompanied by impaired maternal motivation and structural modifications on medium spiny neurons (MSNs) in the NAc. In addition, we examined the possibility that depressive-like behavior and structural outcomes following gestational stress could be reversed by postpartum administration of the antidepressant Citalopram. Pregnant rats were stressed or handled daily between gestation day 7 (GD7) and GD20 and then evaluated for: 1) maternal behavior on postpartum day 2 (PD2), 2) maternal motivation on PD7 using the conditioned place preference paradigm, and 3) depressive-like behavior on PD8 and PD22 using the forced swim test. Brains were collected on PD9 and PD23 for measurement of total dendritic length, arborization, and dendritic spine density of NAc MSNs. Additional groups of stressed and unstressed mothers were treated with Citalopram or saline throughout the postpartum period and depressive-like behavior assessed on PD22. Brains were collected on PD23 to evaluate structural complexity of NAc MSNs as well as pyramidal neurons in the medial prefrontal cortex (mPFC), an area that regulates NAc activity and which shows postpartum structural modifications as a consequence of gestational stress. Our results demonstrate that gestational stress induces persistent depressive-like behavior, deficits in maternal care and maternal motivation as well as reduced dendritic length, branching, and spine density on MSNs in the NAc shell of postpartum rats. Postpartum Citalopram administration ameliorated depressive-like behavior and reversed the structural outcomes of gestational stress in both MSNs in the NAc and pyramidal neurons in the mPFC. Overall these data suggest that altered structural plasticity in brain regions associated with reward and motivation may contribute to attenuated reward processing that accompanies depressive-like behavior during the postpartum period and could represent a potential therapeutic target for PPD.

Disclosures: A. Haim: None. **C. Albin-Brooks:** None. **M.L. Sherer:** None. **E. Mills:** None. **B. Leuner:** None.

Nanosymposium

773. Neuroscience and Psychology of Motherhood

Location: 140A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 773.05

Topic: E.03. Behavioral Neuroendocrinology

Support: AD Society of Canada

Title: Reproductive experience alters the effects of Premarin and surgical menopause on spatial memory and hippocampal plasticity in middle-aged female rats

Authors: *M. M. ROES, C. VAN DEN BRINK, S. LIEBLICH, C. CHOW, L. GALEA; Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The efficacy of hormone replacement therapies (HRTs) as therapeutic agents against age-related cognitive decline in post-menopausal women is controversial. The type of estrogen administered (estradiol v. estrone) and age of treatment initiation influences whether treatment outcome is beneficial, ineffective, or deleterious to the aging brain. HRT outcome may also depend on a female's history of pregnancy and mothering (reproductive experience). Reproductive experience has persisting effects on the aging hippocampus, altering performance on hippocampus-dependent tasks and the sensitivity of the female brain to different types of estrogens long after offspring are weaned. Estrogen treatments may therefore affect cognition and brain health in aging females differently depending on reproductive experience and hormonal status. The current study aimed to elucidate the effects of reproductive experience and ovarian hormone status on the cognitive and neuroplastic effects of Premarin, a commonly-prescribed estrone-based therapy, in middle-aged female rats. Primiparous (one reproductive experience) or nulliparous (no reproductive experience) rats underwent ovariectomy or sham ovariectomy at 8 months of age. At 13 months of age, rats received daily subcutaneous doses of Premarin or vehicle for 21 days. In the final week of hormone administration, rats were tested for hippocampus-dependent learning using the Morris Water Maze (MWM). Preliminary findings reveal that chronic Premarin administration enhanced MWM performance in nulliparous rats but impaired performance in primiparous rats. In addition, reproductive experience increased the expression of the immediate early gene (zif268) in the hippocampus in response to spatial memory, and this effect was moderated by ovarian hormone status and hippocampal region. We predict that reproductive experience will also influence cell survival in the dentate gyrus of the hippocampus and the activation of adult-generated hippocampal cells in response to spatial memory. Our findings suggest that reproductive experience and hormonal status alter the aging female brain's response to Premarin. Tailoring HRT treatment to women's reproductive history may be important in promoting healthy brain aging in women. Funded by AD Society of Canada to LAMG.

Disclosures: **M.M. Roes:** None. **C. van den Brink:** None. **S. Lieblich:** None. **C. Chow:** None. **L. Galea:** None.

Nanosymposium

773. Neuroscience and Psychology of Motherhood

Location: 140A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 773.06

Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant DC 009910

NIMH Grant MH091451

NARSAD Young Investigator Award from the Brain & Behavior Research Foundation

University of Michigan

Title: Mother-to-infant transmission of trauma through social fear learning: Behavioral, neural and endocrine mechanisms

Authors: *J. DEBIEC[1], R. M. SULLIVAN[2];
[1]The Mol. & Behavioral Neurosci. Inst., Univ. of Michigan, Ann Arbor, MI; [2]Nathan Kline Inst., Orangeburg, NY

Abstract: Background: Parental fear and trauma may be transmitted to their offspring. Despite existing evidence of this transmission the neuroendocrine mechanisms underlying the social transfer of fear remain unknown. We have recently proposed a rat model of the mother-to-infant social transmission of fear. In our model, infant rats exposed to their mother expressing fear in response to the previously conditioned olfactory conditioned stimulus, CS, acquire lasting aversive responses to this CS. We observed that an exposure to the maternal alarm odor alone paired with the neutral odor was sufficient to produce in pups fear responses to this odor. We found that this mother-to-infant transmission of fear is associated with increased activity of the infant's amygdala. Here, we explored the role of the amygdala and CORT in the intergenerational transmission of fear. Methods: Experiment 1: Pups with bilateral intra-lateral and basal amygdala (LBA) cannulae were infused either with a GABA A receptor agonist muscimol (n=5) or saline (n=5) prior to exposure to mother expressing fear in response to the CS odor. On the following day, pups were tested to the maternal CS using a Y-maze test in which a pup has to choose between the CS and a neutral odor. Experiment 2: Pups were exposed either to the odor of the frightened mother (n=12) or the odor of the unfrightened mother (n=8). Subsequently, pups' serum CORT levels were assessed using radioimmunoassay. Experiment 3: Pups received systemic injections of the CORT synthesis blocker metyrapone (n=9) or saline (n=7) prior to exposure the mother expressing fear in response to the CS odor. Results:

Experiment 1: The t-test revealed a significant effect of treatment, $t(8) = 4.714$, $p < 0.02$, with the 'Muscimol' group, compared to the 'Control' group, failing to demonstrate the decreased number of the CS-odor choices indicative of learning deficit. Experiment 2: Pups exposed to the odor of the 'Frightened' vs. 'Unfrightened' mother displayed significantly higher levels of CORT $t(16.18) = 2.133$; $p < 0.05$. Experiment 3: Pups from the 'Metyrapone' group failed to show the learned odor aversion ($t(14)=5.465$, $p < 0.002$). Conclusions: We have demonstrated here that the LBA is critical for the social intergenerational transmission of fear. We have shown that this transmission of maternal fear is associated in infants with increased CORT levels and that CORT blockade prevents mother-to-infant transfer of fear. If confirmed in humans, our findings may help to develop novel preventive and therapeutic approaches aimed at disruption of this early intergenerational transmission of trauma.

Disclosures: J. Debiec: None. **R.M. Sullivan:** None.

Nanosymposium

773. Neuroscience and Psychology of Motherhood

Location: 140A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 773.07

Topic: E.03. Behavioral Neuroendocrinology

Support: NIH P01DA022446

NIH R03DA034863

Title: Maternal cocaine reduces frequency of dopamine release events in the nucleus accumbens of rat mothers

Authors: *D. L. ROBINSON[1,2], D. L. ZITZMAN[1], E. H. ANDERSEN[3], T. A. SHNITKO[1], J. M. JOHNS[2,1];
[1]Bowles Ctr. for Alcohol Studies, [2]Dept. of Psychiatry, [3]Curriculum in Neurobio., Univ. of North Carolina, Chapel Hill, NC

Abstract: The mother and infant form a critical dyad to promote the survival of the offspring. The mother exhibits maternal behavior (MB), a complex response to infant cues that is orchestrated by the hormonal and neuropeptide milieu that arises at parturition and continues into the postpartum period. The infant, in turn, displays stimuli that elicit MB. It is well documented in humans and rodents that maternal cocaine exposure during pregnancy results in subsequent

maternal neglect, and new data indicate that cocaine-exposed infants produce stimuli that are distinguishable and perhaps aversive compared to non-exposed infants. In the rodent, mesolimbic dopamine is normally increased during focused maternal care of pups, such as licking and grooming, and disruption of mesolimbic dopamine transmission disrupts MB. Therefore, we hypothesized that reductions in maternal mesolimbic dopamine release contribute to the dysfunctional maternal-infant interactions observed in cocaine-exposed dyads. To create a real-time dopamine profile in non-drug-treated mothers and to test our hypothesis that gestational cocaine treatment blunts dopamine transients during maternal interaction, we used fast-scan cyclic voltammetry with 100-ms and 100-µm sampling to measure individual dopamine release events (aka transients) in mothers on postpartum day (PPD) 1 and, when possible, on PPD2. Dopamine transients in the nucleus accumbens and MB were recorded across 3 epochs each day: a baseline period, for 30m following removal of the litter, and for 30m after the return of her litter. On PPD1, untreated mother rats exhibited the most transients when initially interacting with their pups, fewer when pups were removed, and still fewer when the pups were returned. In contrast, the cocaine-exposed mothers exhibited many fewer transients than untreated mothers at all phases of the experiment, with little variability across epochs. Cocaine-exposed mothers also exhibited more self-directed behaviors and fewer pup-directed behaviors than untreated mothers. A subset of rats was run on PPD2: untreated mothers exhibited higher numbers of dopamine transients across all experimental epochs than were recorded from those rats on PPD1, with no decline as observed on day 1. In contrast, the cocaine-exposed rat that was recorded on PPD2 exhibited fewer dopamine transients than were recorded from that rat on PPD1, suggesting that the cocaine-induced decline persisted at least until the second postpartum day. This study is ongoing, but results to date support the hypothesis that disruption in maternal dopamine signaling contributes to reductions in MB after gestational cocaine exposure.

Disclosures: D.L. Robinson: None. **D.L. Zitzman:** None. **E.H. Andersen:** None. **T.A. Shnitko:** None. **J.M. Johns:** None.

Nanosymposium

773. Neuroscience and Psychology of Motherhood

Location: 140A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 773.08

Topic: E.03. Behavioral Neuroendocrinology

Support: NARSAD YI Award

Title: Disrupted lactation increases depression-like behavior in postpartum rats

Authors: *J. L. WORKMAN, A. R. GOBINATH, S. SOLOMON, C. CHOW, L. A. M. GALEA;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Women are at least twice as likely as men to develop depression and the postpartum period represents a unique time of risk. Approximately 15% of women develop postpartum depression (PPD) and of those women, a significant number also discontinue breastfeeding. It is unclear, however, whether discontinuation of breastfeeding increases the risk for PPD. We hypothesized that disrupted lactation in a rat model would increase depression-like behavior as measured in the forced swim test (FST; a commonly-used and well-validated behavioral test that approximates symptoms of depression). Adult female rats received either thelectomy (surgical removal of teats) or sham surgery and 7 days later, all females were mated with males. Upon parturition, litters were culled to 8 pups and pups were rotated to shams every 12 hours to ensure adequate milk intake. Thus, pup exposure to thelectomized (Thel) females was yoked to that of paired sham females. Maternal behaviors were scored during the first week of the postpartum period. All females were then tested in an open field and FST 3 times throughout the postpartum period. At the end of the postpartum period, blood samples were taken before and after restraint to determine corticosterone responses to a stressor. Lavage samples were taken daily throughout the postpartum period to determine the end of lactational diestrus. Finally, brains were collected and processed for Golgi impregnation. Disrupted lactation elevated immobility in the FST late postpartum and increased locomotor activity early postpartum. Collectively, this suggests that disrupted lactation increases depression-like behavior independently of locomotor activity. Thel females also had a greater percent increase in corticosterone responses after a stressor and spent less time self grooming and nest building than sham females. Finally, Thel females had greater uterine mass (even when controlled for body mass) and had an earlier onset of estrus. Brains are currently being evaluated for spine density and dendritic complexity in the hippocampus and prefrontal cortex. This research will be instrumental in understanding emotional and neural changes that occur in women who discontinue breastfeeding early postpartum.

Disclosures: J.L. Workman: None. **A.R. Gobinath:** None. **S. Solomon:** None. **C. Chow:** None. **L.A.M. Galea:** None.

Nanosymposium

773. Neuroscience and Psychology of Motherhood

Location: 140A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 773.09

Topic: E.03. Behavioral Neuroendocrinology

Support: Supported by 2013 NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation and a NICHD grant HD073710 awarded to MP

Title: Impairments in cognitive flexibility are associated with deficits in parenting in animal models of postpartum depression

Authors: *M. PEREIRA[1], V. KHANNA[2], M. W. SHIFLETT[2], J. I. MORRELL[1];
[1]Rutgers, The State Univ. of New Jersey, CMBN, NEWARK, NJ; [2]Rutgers, The State Univ. of New Jersey, Psychology, NEWARK, NJ

Abstract: Postpartum depression is a serious psychiatric condition that has deleterious effects on the mother, and poses a risk for the mother-infant relationship and ultimately the infant's development. Deficits in cognitive functions regulated by the prefrontal cortex, including impairments in behavioral flexibility, are major clinical features central to postpartum depression and likely contribute to deficits in parenting. The present study used Wistar Kyoto (WKY) mother rats, an animal model of depression which we have developed to examine the postpartum disorder (Pereira et al. 2012), to investigate the neurobiological substrate underlying the relationship between depressive-like cognitive deficits and parenting disturbances. WKY and Sprague Dawley (SD) postpartum females were examined for their ability to flexibly adapt responding based on rule change in an operant set-shifting task, in combination with detailed analysis of maternal behavior. Consistent with clinical observations of cognitive dysfunction in depressed human mothers, WKY mothers exhibited performance deficits on the response reversal and strategy set-shifting components of the operant set-shifting task, indicative of impaired cognitive flexibility. In addition to these cognitive deficits, WKY mothers exhibited severe disturbances in maternal behavior that resembled the behavioral effects of medial prefrontal cortex (mPFC) dopamine antagonism in control SD postpartum females. In WKY mothers, systemic administration of the A2A adenosine antagonist MSX-3 attenuated deficits on strategy set-shifting, with WKY mothers requiring significantly fewer trials to shift their response between rules compared with vehicle, and ameliorated corresponding deficits in maternal responding. Cross-fostering experiments revealed that cognitive and maternal behavior deficits were partially attenuated when WKY females were raised by SD mothers, whereas the opposite was true for SD females raised by WKY mothers. Taken together, these results suggest that deficits in cognitive functions mediated by mPFC coincide with deficits in parenting, revealing a potential neurobiological mechanism by which maternal behavior is compromised in postpartum depression.

Disclosures: **M. Pereira:** None. **V. Khanna:** None. **J.I. Morrell:** None. **M.W. Shiflett:** None.

Nanosymposium

773. Neuroscience and Psychology of Motherhood

Location: 140A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:30 PM

Presentation Number: 773.10

Topic: E.03. Behavioral Neuroendocrinology

Support: FIS/IMSS/PROT/G13/1224

Title: Stress exposure in different life periods result in similar behavioral and neuroendocrine alterations in postpartum female rats: Comparison between two animal models of postpartum depression

Authors: *L. TORNER[1], N. Y. GARCIA-VALENZUELA[1], B. MORENO-CARRANZA[2], C. CLAPP[2];
[1]Neuroendocrinology, Inst. Mexicano Del Seguro Social, Morelia, Mexico; [2]Inst. de Neurobiologia, Univ. Nacional Autónoma de México, Juriquilla, Queretaro, Mexico

Abstract: The neurobiological systems of the female brain are activated during pregnancy to prepare the female to cope successfully with motherhood. This is important for the optimal development of the offspring and for the wellbeing of the mother. Stress exposure modifies maternal adaptations increasing the risk of the mother to develop emotional and behavioral alterations during the postpartum period. Our aim was to compare two different times of stress exposure (a) as adults during pregnancy (adult stress) or (b) as embryos, during prenatal development (gestational stress) on the maternal and depressive - like behaviors of lactating rats and their neuroendocrine expression of relevant peptides (Oxytocin; OXT) or its receptors (Prolactin receptors; PRL-R). Three groups of female Sprague Dawley rats were used: (a) Adult stress group (AS): lactating females subjected to 1h immovilization stress from days 11 to 20 of pregnancy under bright lights. (b) Gestational stress group (GS): lactating females subjected to stress during prenatal development (female offspring of AS mothers). (c) Control group (CL): lactating females never subjected to stress procedures. Lactating rats from all groups were sacrificed at day 2 postpartum (PP) and the hypothalamy were extracted. Total RNA was isolated, retrotranscribed to cDNA, and amplified using real time PCR with specific nucleotides for OXT, vasopressin (AVP), corticotrophin releasing hormone (CRH) and PRLR. The maternal behavior was scored during days 1 and 2 PP in additional groups (6 observations/day, 30 min periods every 3 min; 9 to 19h). Pup retrieval test (10 min in a fresh cage) was evaluated on day 3PP, and depressive - like behavior was evaluated at day 4PP with the forced swimming test. A significant decrease in OXT, AVP, and CRH expression was observed in both AS and GS groups, however, PRLR expression was higher in AS and GS groups compared with CL group.

EC and EP rats showed a deficient maternal behavior and a depressive - like behavior compared to CL rats. Conclusions: Regardless of the life period of stress exposure, convergence of neuroendocrine and behavioral alterations is observed in lactating female rats during the early postpartum period. (Support: grant FIS/IMSS/PROT/G13/1224 from IMSS).

Disclosures: L. Torner: None. **N.Y. Garcia-Valenzuela:** None. **B. Moreno-Carranza:** None. **C. Clapp:** None.

Nanosymposium

774. Respiratory Neurobiology

Location: 150A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:15 PM

Presentation Number: 774.01

Topic: E.04. Autonomic Regulation

Support: NIH HL074011

Title: State-dependent regulation of breathing by the retrotrapezoid nucleus (RTN)

Authors: P. G. BURKE[1], R. KANBAR[2], T. BASTING[1], W. M. HODGES[1], K. E. VIAR[1], R. L. STORNETTA[1], *P. G. GUYENET[3];
[1]Pharmacol., Univ. of Virginia, Charlottesville, VA; [2]Pharmaceut. Sci., Lebanese American Univ., Beyrouth, Lebanon; [3]Pharmacol., Univ. Virginia Sch. Med., Charlottesville, VA

Abstract: RTN is a bilateral cluster of putative central respiratory chemoreceptors (CCRs) located at the ventral surface of the rostral medulla oblongata. The exact role of this nucleus in respiratory control is unclear. Depending on the source, RTN is said to regulate breathing selectively during the waking state, the nucleus does or does not contribute to resting ventilation or it regulates predominantly active expiration. These interpretations are at odds with mouse genetics studies which suggest that the congenital absence of RTN could explain the severe apnea present in congenital central hypoventilation syndrome (CCHS) only during sleep. Here we use optogenetics to reinvestigate the contribution of RTN neurons to breathing in conscious rats whose state of vigilance was continuously monitored (EEG and neck EMG). RTN neurons were unilaterally transfected with ChR2 or bilaterally transfected with ArchT (photoactivatable proton pump) using lentiviral vectors (PRSx8-ChR2 LVV or PRSx8-ArchT LVV). In anesthetized rats RTN neurons could be instantly and reversibly activated (ChR2) or silenced (ArchT) by light. RTN silencing (10 s) in 6 conscious rats produced instant reductions of breathing frequency (fR, -22 ± 5 %) and tidal volume (Vt, -24 ± 5 %). The magnitude of these

effects was the same during non-REM sleep and quiet waking (QW). During REM sleep, RTN inhibition had no effect on fR but still reduced Vt (-13 ± 4%). Optogenetic stimulation of RTN in 5 conscious rats (ChR2) increased fR and Vt about equally during QW and non-REM sleep but did not change fR during REM sleep. In conclusion: 1. During eupnea RTN regulates both fR and Vt in a CO2-dependent manner. 2. During REM sleep RTN neurons regulate Vt but no longer regulate fR. 3. Our observations support prior suggestions that the congenital absence of RTN could cause severe hypoventilation during non-REM sleep.

Disclosures: **P.G. Burke:** None. **R. Kanbar:** None. **T. Basting:** None. **W.M. Hodges:** None. **K.E. Viar:** None. **R.L. Stornetta:** None. **P.G. Guyenet:** None.

Nanosymposium

774. Respiratory Neurobiology

Location: 150A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:15 PM

Presentation Number: 774.02

Topic: E.04. Autonomic Regulation

Support: NIH HL074011

Title: Hypoxia silences the retrotrapezoid nucleus (RTN) in conscious rats

Authors: ***I. C. WENKER**[1], T. BASTING[1], P. G. R. BURKE[1], R. KANBAR[2], K. E. VIAR[1], R. L. STORNETTA[1], P. G. GUYENET[1];
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Abstract: The notion that hypoxia silences central respiratory chemoreceptors (CCRs) as a result of respiratory alkalosis predates our understanding of the cellular/molecular nature of these sensors by many decades (Gesell R, Lapides J, Levin M. Am J Physiol 130:155-170,1940). RTN is a bilateral cluster of putative CCRs located at the ventral surface of the rostral medulla oblongata. Here we test Gesell's theory by monitoring how much RTN neurons contribute to breathing in conscious adult Sprague-Dawley rats exposed to normoxia or hypoxia (15 and 12% FiO2, balance N2). RTN neurons were bilaterally transduced with ArchT (light-activated proton pump) using a lentiviral vector. In 3 anesthetized rats, ArchT photoactivation (10s, 5 mW) instantly and reversibly silenced RTN neurons. In conscious rats (N=9), hypoxia stimulated breathing moderately (27±7 % increase in minute-volume, MV, at 12% FiO2) and caused substantial respiratory alkalosis (pHa 7.63 at 12% FiO2 vs. 7.44 in normoxia). When the rats

were either quietly awake or in non-REM sleep, silencing ArchT-transduced RTN neurons bilaterally reduced the breathing frequency (fR) by -19±3 and -22±5 %, respectively, and MV by -38±7 and -36±3 % (N=9). The inhibition of fR was halved in 15% O2 hypoxia and eliminated at 12% O2. Adding 3% CO2 to the 12% FiO2 stimulated breathing and restored the hypopnea elicited by optogenetic inhibition of RTN neurons. Administration of the carbonic anhydrase inhibitor acetazolamide had the same effect. ChR2-mediated optogenetic activation of RTN increased breathing to a similar extent in 21, 15 or 12% FiO2 indicating that synaptic transmission between RTN and the respiratory network was unimpeded by hypoxia. In conclusion: 1. RTN is virtually silenced by hypoxia, most likely because of the associated plasma/brain alkalization, 2. During hypoxia, the carotid bodies stimulate breathing via pathways that bypass RTN, 3. Acetazolamide increases breathing during hypoxia by reactivating RTN and, 4. During hypoxia RTN neurons respond precisely as expected from CCRs.

Disclosures: I.C. Wenker: None. **T. Basting:** None. **P.G.R. Burke:** None. **R. Kanbar:** None. **K.E. Viar:** None. **R.L. Stornetta:** None. **P.G. Guyenet:** None.

Nanosymposium

774. Respiratory Neurobiology

Location: 150A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:15 PM

Presentation Number: 774.03

Topic: E.04. Autonomic Regulation

Support: FAPESP

CNPq

NIH

Title: Regulation of breathing by purinergic mechanisms in the retrotrapezoid nucleus in conscious rats

Authors: *T. S. MOREIRA[1], B. F. BARNA[1], D. K. MULKEY[3], A. C. TAKAKURA[2]; [1]Physiol. and Biophysics, [2]Pharmacol., Univ. of Sao Paulo, Sao Paulo, Brazil; [3]Physiol. and Neurobio., Univ. of Connecticut, Storrs, CT

Abstract: Central and peripheral chemoreceptors sense changes in CO2/H+ and/or O2 and communicate this information to cardiorespiratory centers to regulate breathing and sympathetic outflow to ensure adequate ventilation-perfusion matching in tissues. Despite the importance of

this reflex, the neurotransmitter basis for integration of this information in the brainstem is unclear. It has been shown that excitatory drive from peripheral chemoreceptors is relayed through the retrotrapezoid region (RTN) located in the ventrolateral medulla, a region also known to contribute to central chemoreception. Evidence also suggests that purinergic signaling in this region can influence both respiratory and sympathetic drive, possibly by activation of purinergic receptors. Therefore, our main goal was to elucidate the involvement of purinergic receptors for central and peripheral chemoreceptor integration at the level of the RTN region in unanesthetized rats. Male Wistar rats (270-300g, n = 5/group) with bilateral stainless steel cannula implanted into the RTN were used. Bilateral injection of pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonate (PPADS, a P2 receptor blocker - 5 mM) decreased the ventilatory (Ve) response (979 ± 91, vs. saline: 1196 ± 138 ml/kg/min) to hypercapnia (7% CO2). However, PPADS did not change the Ve response (849 ± 118, vs. saline: 818 ± 82 ml/kg/min) to hypoxia (8% O2). Inhibition of the P2Y1 receptors in the RTN with bilateral injection of MRS2179 (100 μM) had no effect on respiratory changes elicited by hypercapnia or hypoxia. Both bilateral injections of PPADS and MRS2179 did not change the cardiovascular parameters. These results support the possibility that purinergic signaling in the RTN region is important to central chemoreflex in unanesthetized animals.

Disclosures: **T.S. Moreira:** None. **B.F. Barna:** None. **D.K. Mulkey:** None. **A.C. Takakura:** None.

Nanosymposium

774. Respiratory Neurobiology

Location: 150A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:15 PM

Presentation Number: 774.04

Topic: E.04. Autonomic Regulation

Support: DGAPA-UNAM IB200212

CONACyT 151261

CONACyT 181323

NIRG-11-205443

Fellowship from Conacyt to Flores-Martínez E

Fellowship from Conacyt to Nieto-Posadas A

Fellowship from Conacyt to Lorea-Hernández J.J.

Title: Change in network connectivity during gasping generation in hypoxia: Prevention by a metabolic intermediary

Authors: *E. FLORES[1,2], A. NIETO-POSADAS[2], J. LOREA-HERNÁNDEZ[2], A. RIVERA-ANGULO[2], J. PÉREZ-ORTEGA[3], J. BARGAS[3], F. PEÑA-ORTEGA[2];
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Abstract: The neuronal circuit in charge of generating the respiratory rhythms, localized in the pre-Bötzinger complex (preBötC), is configured to produce eupnea during normoxia and reconfigures to produce gasping during hypoxic conditions. The mechanisms involved in such reconfiguration have been extensively investigated by cell-focused studies but the actual changes at the network level remain elusive. Since a failure to generate gasping has been linked to Sudden Infant Death Syndrome, the study of gasping generation, as well as the pharmacological approaches to promote it, may have clinical relevance. Here, we study the changes in network dynamics and circuit reconfiguration that occur during the transition to fictive-gasping generation in the brainstem slice preparation by means of recording the preBötC with multi-electrode arrays and assessing correlated firing among respiratory neurons or clusters of respiratory neurons (multiunits). We wanted to test whether the respiratory network reconfiguration in hypoxia involves changes either in the number of active respiratory recording nodes, changes in the number of functional connections among nodes or changes in the strength of these connections. Moreover, we tested the influence of isocitrate, an intermediate of the Krebs cycle that has recently been shown to promote breathing, upon the configuration of the preBötC circuit during normoxia and on its reconfiguration during hypoxia. We found that, in contrast with previous suggestions based on cell-focused studies, the number and the overall activity of respiratory neurons change slightly during hypoxia. Alternatively, hypoxia induces a reduction in the strength of functional connectivity within the circuit without a reduction the number of connections. Isocitrate prevented the reduction in the strength of circuit interactions during hypoxia while increasing the strength of network connectivity. In conclusion, we provide an overview of the configuration of the respiratory network under control conditions and how it is reconfigured during fictive-gasping. Additionally, our data support the use of isocitrate as a strategy to favor respiratory rhythm generation under normoxia and to prevent some of the changes in the respiratory network under hypoxic conditions.

Disclosures: **E. Flores:** None. **A. Nieto-Posadas:** None. **J. Lorea-Hernández:** None. **A. Rivera-Angulo:** None. **J. Pérez-Ortega:** None. **J. Bargas:** None. **F. Peña-Ortega:** None.

Nanosymposium

774. Respiratory Neurobiology

Location: 150A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:15 PM

Presentation Number: 774.05

Topic: E.04. Autonomic Regulation

Support: Intramural Research Program of NINDS, NIH

Title: Functional role of pre-Bötzinger complex excitatory neurons in respiratory rhythm generation: Optogenetic studies

Authors: *H. KOIZUMI, N. KOSHIYA, R. ZHANG, B. MOSHER, J. C. SMITH;
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Abstract: The pre-Bötzinger complex (pre-BötC) has long been identified as a core circuitry for respiratory rhythm generation in mammals. The pre-BötC contains heterogeneous populations of excitatory and inhibitory interneurons, and it is generally assumed that the excitatory (glutamatergic) neurons are the substrate for inspiratory rhythm generation but this has been difficult to demonstrate experimentally in the absence of techniques for cell population-specific manipulation of excitatory neuron activity. We have applied optogenetic approaches to investigate the population-specific role of pre-BötC glutamatergic neurons in respiratory rhythm generation in neonatal brainstem slices in vitro and in the functionally more intact juvenile/adult perfused brainstem-spinal cord preparations in situ. We established double-transgenic mouse models for population-specific photoexcitation or inhibition of pre-BötC glutamatergic neurons by crossing Cre-driver mouse lines for glutamatergic neurons (based on the vesicular glutamate transporter 2, VgluT2 promoter) with Cre-dependent optogenetic strains (Archaerhodopsin-3, Arch for inhibition or Channelrhodopsin-2, ChR2 for excitation of targeted neurons). The results from VgluT2-Arch mice showed that bilateral optical inhibition of pre-BötC glutamatergic neuron population (continuous orange laser, 593 nm, 10 mW each) caused rapid and reversible cessation of respiratory rhythm in both in vitro and in situ preparations, indicating that pre-BötC glutamatergic neurons are essential for rhythm generation. Step-wise inhibition of pre-BötC glutamatergic neuron population caused slowing of the respiratory rhythm as a function of the applied laser light intensity (2-10 mW), while in VgluT2-ChR2 mice bilateral optical excitation of pre-BötC glutamatergic neuron population (pulsed blue laser, 473 nm, 10 msec, 20 Hz, 2-10 mW) increased the frequency of respiratory rhythm in both in vitro and in situ preparations. The results are consistent with the concept that pre-BötC glutamatergic neurons are the excitatory kernel for inspiratory rhythm generation in the respiratory network both in vitro and more intact in situ conditions.

Disclosures: **H. Koizumi:** None. **N. Koshiya:** None. **R. Zhang:** None. **B. Mosher:** None. **J.C. Smith:** None.

Nanosymposium

774. Respiratory Neurobiology

Location: 150A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:15 PM

Presentation Number: 774.06

Topic: E.04. Autonomic Regulation

Support: NIH Grant R01 NS069220

NSF Grant 1021701

Title: Computational modeling of the respiratory system: Focus on hypercapnia and active expiration

Authors: Y. I. MOLKOV[1], N. A. SHEVTSOVA[2], C. PARK[3], A. BEN-TAL[4], J. C. SMITH[5], J. E. RUBIN[3], *I. A. RYBAK[2];
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Abstract: We have developed a computational model of the closed-loop respiratory system. The model describes the brainstem respiratory network controlling the pulmonary subsystem that includes lung biomechanics and gas (O_2 and CO_2) exchange and transport. The lung subsystem provides two types of feedback to the neural subsystem: a mechanical one from pulmonary stretch receptors and a chemical one from central chemoreceptors responding to CO_2 concentration in the brainstem. The neural component of the model simulates the pontine-medullary respiratory network that includes several interacting respiratory neuron types within the Bötzinger and pre-Bötzinger complexes as well as the retrotrapezoid nucleus/parafacial respiratory group (RTN/pFRG) representing the central chemoreception module targeted by chemical feedback. The RTN/pFRG compartment contains an independent neural generator that is activated at an increased CO_2 level and controls the abdominal motor output. The lung volume in the pulmonary subsystem is controlled by two pumps, a major one driven by the diaphragm and an additional one activated by abdominal muscles and involved in ventilation during active expiration. Our study represents the first attempt to model the transition from quiet breathing to

breathing with active expiration involving RTN/pFRG neural and abdominal motor activity. The model reproduces multiple experimental data on the effects of various perturbations and stimulations applied to mechanical and chemical feedbacks. Our simulations show that the closed-loop respiratory control system switches to active or forced expiration when increases in breathing rate and phrenic amplitude no longer provide sufficient ventilation. The model can be used for simulation of closed-loop control of breathing under different conditions including respiratory disorders.

Disclosures: Y.I. Molkov: None. **N.A. Shevtsova:** None. **C. Park:** None. **A. Ben-Tal:** None. **J.C. Smith:** None. **J.E. Rubin:** None. **I.A. Rybak:** None.

Nanosymposium

774. Respiratory Neurobiology

Location: 150A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:15 PM

Presentation Number: 774.07

Topic: E.04. Autonomic Regulation

Support: ANR GenResp Grant to GF and JFB

Team FRM Grant to GF

FRM PhD fellowship to PLR

NeRF PhD fellowship to PLR

Title: The retrotrapezoid nucleus (RTN) is essential for CO2 chemoception

Authors: P.-L. RUFFAULT[1], J.-F. BRUNET[2], C. GORIDIS[2], *G. FORTIN[3];
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Abstract: Breathing is a spontaneous, rhythmic motor behavior critical for maintaining O2, CO2 , and pH homeostasis. The neural bases underlying the respiratory chemoreflex that control ventilation in response to deviations in arterial or brain PCO2 or pH are still debated. The retrotrapezoid nucleus (RTN), a collection of neurons located close to the medullary surface ventral and immediately caudal to the facial nucleus (nVII), has been postulated to play a critical role in the central CO2 response, but the part it plays in the respiratory chemoreflex is still controversial. To assess the requirement for RTN neurons in the respiratory chemoreflex, we

applied a recombinase-based intersectional genetic strategy to impair its development in mice. To target the RTN, we exploited the fact that only two groups of cells in the brain co-express the Phox2b and Atoh1 transcription factors or descend from precursors that have expressed them: one surrounding nVII, mostly RTN neurons, and one surrounding the trigeminal motor nucleus (periV neurons). We measured the fictive respiratory responses to acidification or the ventilatory responses to an increase in inspired CO2, respectively, in brainstem-spinal cord preparations of embryos and in neo- and post-natal animals. Invalidation of either Atoh1 in Phox2b+ or of Phox2b in Atoh1+ cells resulted in compound slower rhythm and elimination of the CO2/pH responses in all cases. We show that the disruption of glutamatergic transmission in Atoh1+/Phox2b+ cells led to a similar outcome and that impairment of periV neuronal activity is unlikely to cause the respiratory defect. These findings strongly suggest that the RTN is absolutely essential for the activation of breathing by increased CO2 or low pH.

Disclosures: **P. Ruffault:** None. **J. Brunet:** None. **C. Goridis:** None. **G. Fortin:** None.

Nanosymposium

774. Respiratory Neurobiology

Location: 150A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:15 PM

Presentation Number: 774.08

Topic: E.04. Autonomic Regulation

Support: The Hillcrest Foundation

NHMRC app1028283

Skipper Early Career Travel Award

Title: Effects of acute (systemic and central) and chronic methamphetamine on respiratory, metabolic and cardiovascular tonic and reflex function

Authors: *S. F. HASSAN[1], T. WEARNE[2], C. RADFORD[1], J. L. CORNISH[2], A. K. GOODCHILD[1];
[1]Australian Sch. of Advanced Med., Sydney, Australia; [2]Neuropharm. Lab., Sydney, Australia

Abstract: The effects of acute and chronic methamphetamine (METH) on behaviour are well described. Likewise, METH is widely reported to elicit general sympathetic activation. Despite observations that chronic abuse results in alterations to central monoaminergic transmission and neuronal toxicity at sites critical for autonomic control, both the acute and chronic consequences

of METH on autonomic and respiratory tonic and reflex function, and, whether these changes are mediated by central or peripheral mechanisms are still largely unknown. Therefore, the first aim was to describe comprehensively the respiratory, metabolic and cardiovascular effects of acute METH administered systemically and intracerebroventricularly (ICV) in urethane-anaesthetized male Sprague-Dawley rats. Secondly, we investigated the effects of a regime of chronic METH that evokes behavioural sensitization. Acute METH evoked dose dependent increases in central inspiratory drive ($5mgkg^{-1}$: 182±43%; n=5) and frequency ($5mgkg^{-1}$: 14±3bpm; n=5), which were not dependent on METH-evoked increases in expired CO2 levels. A significant dose dependent increase in brown adipose tissue temperature ($5mgkg^{-1}$: 3.9±0.5°C; n=6) demonstrates that a drive in non-shivering thermogenesis contributes to METH induced hyperthermia. Unexpectedly, METH evoked minor effects on blood pressure and sympathetic outflows to lumbar and splanchnic beds. Furthermore, METH modified adaptive cardiorespiratory reflex function to challenge with hypoxia, hypercapnia and baroreceptor unloading. ICV METH evoked similar changes in all parameters modified by systemic administration. Chronic METH, however, did not change resting respiratory, metabolic or cardiovascular outflows, cardiorespiratory reflexes or peak responses to a challenge dose of METH in anaesthetized rats. However, respiratory and cardiac components of the hypercapnic and sympathetic baroreflex were altered in all METH treated rats. These findings significantly extend the work of previous studies to demonstrate actions at central respiratory pathways and those involved in thermogenic drive. This study serves to highlight that a single dose of METH can impact basic homeostatic systems and protective functions and its chronic effects deserve future investigation.

Disclosures: S.F. Hassan: None. **T. Wearne:** None. **C. Radford:** None. **J.L. Cornish:** None. **A.K. Goodchild:** None.

Nanosymposium

774. Respiratory Neurobiology

Location: 150A

Time: Wednesday, November 19, 2014, 1:00 PM - 3:15 PM

Presentation Number: 774.09

Topic: E.04. Autonomic Regulation

Support: KAKENHI (25430012)

Title: Riluzole depresses burst generation of pre-inspiratory neurons in the rostral medulla of the brainstem-spinal cord preparation from newborn rats

Authors: *S. LIN[1], H. ONIAMRU[2];
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Abstract: The contributions of ionic currents play a key role in elucidating how the respiratory rhythm is generated in the brainstem. Riluzole is one of the therapeutic agents for amyotrophic lateral sclerosis (ALS) and is also known as a persistent sodium channel blocker. In the present study, we examined effects of riluzole on pre-inspiratory (Pre-I) and inspiratory (Insp) neurons in the rostral medulla as well as on the 4th cervical ventral root (C_4)-inspiratory activities in the in vitro brainstem-spinal cord preparations from newborn rats. The experimental protocols used in this study were approved by the Institutional Animal Care and Use Committee of Showa University. Preparations were isolated from postnatal day 0 (P0) to P3 Wistar rats and were superfused at a rate of 3.0 ml/min with the following artificial cerebrospinal fluid (in mM): 124 NaCl, 5.0 KCl, 1.24 KH_2PO_4, 2.4 $CaCl_2$, 1.3 $MgCl_2$, 26 $NaHCO_3$ and 30 glucose, equilibrated with 95% O_2 and 5% CO_2, pH 7.4, at 25-26^oC. The rate of C4 inspiratory burst was inhibited in a dose-dependent manner (1-200 μM) after 15 min application of riluzole. The inhibitory effects of riluzole were irreversible especially in higher doses. In addition, riluzole had a tendency to prolong C_4 burst duration but gradually shorten after washout. Results of membrane properties indicated that riluzole caused a strong reduction in the drive potential of Pre-I neurons (but not Insp neurons) and the reduced drive potential did not restore after washout. The burst patterns of inspiratory neurons and C_4 inspiratory activity progressively changed into episodic pattern in which one burst consisted of 3-9 short separate bursts, typically observed during washout period. Induction of action potentials in response to membrane depolarization by current injection significantly depressed during washout period. Our findings indicated that the burst generation of Pre-I neurons is more sensitive to riluzole than inspiratory burst generation and thus suggested an important role of persistent sodium channels in the burst generation of Pre-I neurons.

Disclosures: S. Lin: None. **H. Oniamru:** None.

Nanosymposium

775. Long-Term Memory: Medial Temporal Lobe

Location: 143A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 775.01

Topic: F.01. Human Cognition and Behavior

Support: DAAD postdoctoral fellowship grant

Title: Predicting the integration of older and newer experiences

Authors: *F. R. RICHTER, A. J. H. CHANALES, B. A. KUHL;
Dept. of Psychology, New York Univ., New York, NY

Abstract: Our current experience often overlaps with past experience, which can trigger the reactivation of older memories. While reactivation of older memories has the potential to distract from new encoding, *integration* of reactivated memories with current experience could reduce tradeoffs between encoding and retrieval. Here we assessed whether the process of integration can be distinguished_using multi-voxel fMRI pattern analysis_from encoding and retrieval and, in turn, whether fMRI-based evidence of integration during new learning can be used to predict the subsequent ability to remember across events. During fMRI scanning, human subjects (n = 21) studied associations between words and pictures (faces, scenes, objects). After acquiring initial word-picture associations (A-B pairs), they completed a critical *new learning* phase in which previously learned words were paired with new pictures (A-C pairs). Each A-C pair was briefly presented (2 s) and immediately followed by a cue instructing subjects to either recall the old (A-B) association (*retrieve* condition), focus on encoding the new (A-C) association only (*encode* condition), or to recall the old association and integrate it with the new association (*integrate* condition). After exiting the scanner, subjects completed a surprise test which assessed their ability to recall B terms when presented with C terms (i.e., an integration test). Behavioral data indicated that instructions to integrate improved memory on the integration test, confirming that subjects internally modulated mnemonic processing in-line with instructions. Pattern classification analyses applied to the fMRI data from the new learning phase indicated that the ability to decode the category of the B and C items (face vs. scene vs. object) was strongly related to the task manipulation (to encode, retrieve, or integrate). Of central interest, we also trained an across-subject pattern classifier to decode, on a trial-by-trial basis, the task that subjects were performing (encode vs. retrieve vs. integrate), a dimension that was orthogonal to visual category. Critically, the integrate condition could be discriminated from both the encode and retrieve conditions (p's < .005). Moreover, classifier-based evidence for integration_but not evidence for retrieval or encoding_predicted subsequent performance on the integration test (an effect that held true when controlling for the task subjects were instructed to perform). Finally, we consider the potential for using a pattern classifier trained from this data set to predict integration in an independent data set in which subjects were never explicitly instructed to integrate.

Disclosures: F.R. Richter: None. **A.J.H. Chanales:** None. **B.A. Kuhl:** None.

Nanosymposium

775. Long-Term Memory: Medial Temporal Lobe

Location: 143A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 775.02

Topic: F.01. Human Cognition and Behavior

Support: Swiss National Science Foundation grant 320000-114012

Title: Hippocampus is place of interaction between unconscious and conscious memory retrieval

Authors: *M. A. ZUEST[1], P. COLELLA[1], T. P. REBER[1], S. RUCH[1], P. VUILLEUMIER[2], M. HAUF[3], K. HENKE[1];
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Abstract: Recent experiments suggest that humans can form and later retrieve new semantic relations unconsciously by way of hippocampus - the key structure thought to support conscious relational (episodic) memory. Given that the hippocampus subserves both conscious and unconscious relational encoding/retrieval, we expected the hippocampus to be place of unconscious-conscious interactions. This hypothesis was tested in an fMRI experiment on the interaction between the unconscious retrieval of face-associated occupations and the subsequent conscious retrieval of celebrities' occupations. For subliminal encoding, masked combinations of an unfamiliar face and a written occupation ("actor" or "politician") were subliminally presented. At test, we presented the former subliminal faces again, without occupations and masks, as conscious retrieval cues. We hypothesized that faces would trigger the unconscious reactivation of the associated occupation - actor or politician -, which in turn would facilitate or inhibit the subsequent conscious recollection of a celebrity's occupation. Following the presentation of a former subliminal face, we presented the portrait of a celebrity that participants were required to sort according to "actor" or "politician". Depending on whether the triggered unconscious occupation was congruent or incongruent with the celebrity's occupation, we expected an expedited or retarded conscious retrieval process as reflected in reaction times. Conscious retrieval was expedited in the congruent condition, but there was no effect in the incongruent condition. fMRI data collected during subliminal relational encoding confirmed that the hippocampus was interacting with neocortical semantic storage sites. fMRI data collected at test indicated that the facilitated conscious retrieval of celebrity-associated occupations was related to deactivations in this same network spanning hippocampus and neocortical semantic storage sites. Hence, unconscious retrieval likely preactivated this network, which allowed for a sparing recruitment of additional neural resources to assist conscious retrieval. This finding supports the notion that consciously and unconsciously acquired relational memories are stored in a single, cohesive hippocampal-neocortical memory space.

Disclosures: **M.A. Zuest:** None. **P. Colella:** None. **T.P. Reber:** None. **S. Ruch:** None. **P. Vuilleumier:** None. **M. Hauf:** None. **K. Henke:** None.

Nanosymposium

775. Long-Term Memory: Medial Temporal Lobe

Location: 143A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 775.03

Topic: F.01. Human Cognition and Behavior

Support: NSF CRCNS IIS1207833

NIH NINDS 5R01NS53366

John Templeton Foundation

NIH R01 MH069456

NIH R01 EY021755

NSF GRFP DGE0646086

Title: Rapid learning of complex temporal regularities in the hippocampus: Evidence from fMRI and a neural network model

Authors: *A. C. SCHAPIRO, K. A. NORMAN, N. B. TURK-BROWNE, M. M. BOTVINICK;
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Abstract: Humans quickly and effortlessly learn the structure of their environment. The medial temporal lobe (MTL) seems to play an important role in this rapid statistical learning. For example, we recently found that the neural representations of objects that appeared nearby in time became more similar to each other throughout the MTL (Schapiro, Kustner, & Turk-Browne, 2012, Curr Biol) and that a patient with MTL damage failed to learn such temporal regularities (Schapiro, Gregory, Landau, McCloskey, & Turk-Browne, in press, JOCN). Extending prior work that explored other brain systems (Schapiro, Rogers, Cordova, Turk-Browne, & Botvinick, 2013, Nat Neurosci), we tested whether the MTL's role in rapid statistical learning extends to events defined by more complex forms of structure. Participants were exposed to continuous sequences of stimuli generated by a graph with community structure,

where the strength of transition probabilities and bigram frequencies were uniform and thus not useful for learning the communities. We found that the MTL was sensitive to this complex temporal structure: Neural representations of objects in the same vs. different communities became more similar to each other in the hippocampus (but not MTL cortex). Moreover, the hippocampus was more active within a community vs. at a community boundary. When examining functional connectivity during the task, the hippocampus interacted more with the left IFG--a region with similar representational similarity--within communities, and it interacted more with the mPFC--a region that also detected event boundaries--at the boundaries. These findings suggest that the hippocampus may be a central hub in a network of regions involved in new event learning. On the surface, the sensitivity of the hippocampus to regularities across multiple experiences seems at odds with its known role in one-shot episodic encoding. To investigate this apparent discrepancy, we applied a neural network model of episodic learning in hippocampal subfields (Ketz, Morkonda, & O'Reilly, 2013, PLOS Comput Biol) to the pair and community learning paradigms above. The representations learned by the model matched those observed in the hippocampus for both paradigms, suggesting that the well-established conjunctive learning machinery of the hippocampus may also support statistical learning. The neuroimaging, patient, and modeling results together indicate that the hippocampus plays a critical and general role in rapid learning of novel temporal regularities.

Disclosures: A.C. Schapiro: None. **K.A. Norman:** None. **N.B. Turk-Browne:** None. **M.M. Botvinick:** None.

Nanosymposium

775. Long-Term Memory: Medial Temporal Lobe

Location: 143A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 775.04

Topic: F.01. Human Cognition and Behavior

Support: NIH R01 MH100121-01

NIH NRSA F32MH094085

NSF CAREER

ARO 55830-LS-YIP

NARSAD

Title: Reward representation in the midbrain and medial temporal lobe during motivated encoding

Authors: *D. ZEITHAMOVA, B. D. GELMAN, A. R. PRESTON;
Univ. Texas, Austin, AUSTIN, TX

Abstract: Memory is influenced by motivation, such as a promise of future monetary reward for successfully remembering an event. Prior research has shown that monetary reward enhances activation within dopaminergic midbrain and the medial temporal lobe (MTL), resulting in better memory for events associated with the reward. This enhancement has been proposed to reflect incorporation of the information about reward context into stored memory representations. However, all prior studies have confounded the reward value with the visual features of the reward cue, leaving unanswered whether MTL responses reflect abstract representation of reward value or the visual appearance of the cue. Here we employed a novel reward manipulation to dissociate between these accounts. Participants underwent functional MRI while encoding pairs of objects, with each pair being preceded by a cue indicating the monetary reward the participant would receive if they successfully remembered the pair. Reward cues could represent one of three values (dollar, dime, penny), each presented in one of two visual forms (word, picture) across different trials. After scanning, participants received a cued recall task, which tested their memory for the object pairs. Behaviorally, participants remembered pairs associated with the highest reward (dollar) more often than pairs preceded by low reward cues (dime or penny), irrespective of the visual form of the reward cue. We then tested which aspects of the cue (reward level, visual form) are represented in MTL and midbrain during encoding using multivoxel pattern analysis (MVPA). We performed two different classification analyses: one aiming to decode the reward value from distributed activation patterns and another to decode the visual form of the cue. We found that encoding patterns in early visual cortices differentiated between the visual form of the cues (words vs. pictures) but not the reward value represented by those cues (dollar, dime, penny). In contrast, MTL and midbrain encoding patterns did not distinguish between the visual cues themselves but rather differentiated trials based on their abstract reward value. While dopaminergic midbrain would be expected to represent reward information based on prior research, our findings provide a novel demonstration of abstract representation of reward within MTL. Moreover, our data suggest that contextual representations within the MTL go beyond spatial location to include information about the motivational significance of events, which may in turn facilitate encoding and subsequent remembering.

Disclosures: D. Zeithamova: None. **A.R. Preston:** None. **B.D. Gelman:** None.

Nanosymposium

775. Long-Term Memory: Medial Temporal Lobe

Location: 143A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 775.05

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01-MH094480

Title: Reinstatement of neural patterns during narrative free recall

Authors: *J. CHEN[1], Y. LEONG[2], K. A. NORMAN[2], U. HASSON[2];
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Abstract: When we perceive the external world, we encode part of that experience into memory. During subsequent recall, configurations of brain activity that occurred during the initial perception may be reinstated. Reinstatement can be measured by comparing the spatial patterns of neural activity during encoding to those observed during recall; this method illuminates what regions or networks in the brain are involved in representation of both the encoded and recalled material. Previous studies have relied on simple stimuli and multiple encoding exposures to amplify reinstatement effects. In this study, we present robust encoding-recall neural pattern similarity in a naturalistic task: watching a movie and freely recalling the plot. Seventeen subjects watched a 50-minute movie (BBC's "Sherlock"), and then verbally recounted the story, all while their brain activity was recorded using functional magnetic resonance imaging (fMRI). Recall sessions lasted 22.2 minutes on average (s.d. 9.3) and did not involve any cueing from the experimenter beyond the initial instructions. Neural data from the movie scans were divided into 50 scene segments, and data from the recall scans were divided into matching segments based on the verbal content of the recall. At each voxel, data were averaged across time within segments, separately for encoding and recall. Similarity between spatial activation patterns during the encoding/recall segment was calculated. A multi-voxel pattern analysis performed using a 3 x 3 x 3 voxel searchlight (3 mm voxels) revealed a network of regions in which encoding/recall similarity was statistically significant. These regions included retrosplenial cortex, precuneus, posterior cingulate, lateral prefrontal and medial prefrontal cortices, posterior parahippocampal gyrus, and angular gyrus. This set of regions corresponded closely with the canonical "default mode network". The same network exhibited sensitivity to information over long timescales in our prior studies, and is anatomically and functionally connected to episodic memory structures such as the hippocampus and medial temporal lobe cortices. Furthermore, a finer-grained temporal analysis showed that the temporal order of sub-scene neural patterns during recall significantly matched the order of occurrence during encoding. In summary, our analyses revealed a network of regions in which neural encoding patterns are reinstated during verbal

narrative recall, suggesting that these regions play a role both during the initial processing of the movie as well as during the retrieval of movie-related episodes from memory.

Disclosures: J. Chen: None. **Y. Leong:** None. **U. Hasson:** None. **K.A. Norman:** None.

Nanosymposium

775. Long-Term Memory: Medial Temporal Lobe

Location: 143A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 775.06

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant (F32 MH100904)

NIH Grant (R21 MH091523)

NSF CAREER Award (1056019)

NIH Grant (R01 MH100121)

Title: Episodic reinstatement affects hippocampal and fronto-parietal comparator signals during mnemonic decision making

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Abstract: A fundamental component of decision making is comparing current perceptual input to expectations derived from memory. This function has typically been ascribed to the hippocampus, which is thought to signal when sensory input diverges from mnemonic expectations. However, building expectations relies on memory reinstatement, thought to depend on processes supported by neocortex. For example, episodic memory retrieval has been associated with reinstatement of neural encoding patterns in sensory cortex. The parietal lobe is thought to both facilitate such reactivation and guide attention to internally generated memory representations according to task context. Though these functions are critical to the ultimate comparison process, an empirical link between the reactivation of episodic content and subsequent comparator signatures is yet to be demonstrated. Here, we used multivoxel pattern analysis (MVPA) of fMRI data to quantify trial-by-trial reactivation of episodic content and assess its behavioral and neural impact on subsequent mnemonic decisions. Participants performed a delayed match-to-memory task: after learning face-object and scene-object pairs,

participants were presented with object retrieval cues. After a short delay, a memory probe appeared that was either the associated face/scene (match) or a foil (mismatch). An MVPA classifier was used to measure reactivation of memory content during the delay period. We hypothesized that the speed of probe decisions would be influenced by delay period reactivation. Consistent with this hypothesis, greater reactivation of related content during the delay was associated with faster match decisions. Mismatch trials showed the inverse relationship; greater reactivation preceded slower decisions. We tested the neural prediction that memory reactivation would impact neural comparison signatures during the decision probes. We found that reactivation modulated comparator signals in posterior hippocampus and left inferior frontal gyrus, such that greater delay period reactivation was associated with enhanced mismatch responses during the decision probe. Activation in left angular gyrus showed the opposite relationship; greater reactivation was associated with reduced mismatch responses. These results highlight the important role episodic reinstatement plays in mnemonic comparison processes mediated by the hippocampus. Moreover, our results are consistent with the notion that lateral parietal regions serve as an episodic buffer for retrieval, while lateral prefrontal regions may resolve competition among discrepant sources of evidence.

Disclosures: M.L. Mack: None. **A.R. Preston:** None.

Nanosymposium

775. Long-Term Memory: Medial Temporal Lobe

Location: 143A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 775.07

Topic: F.01. Human Cognition and Behavior

Support: UMD DRI seed grant

UMD MNC seed grant

NSF GRFP

Title: Context-dependent modulation of amygdala-prefrontal networks in early childhood

Authors: *E. REDCAY, K. RICE, T. RIGGINS;
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Abstract: Amygdala and medial prefrontal (mpfc) networks are critical for social attention and emotion processing. Though significant advances in social-emotional processing occur in early

childhood, very little work has examined early development of amygdala-mpfc circuits. Previous studies examining amygdala-mpfc connectivity during rest in children have reported that prior to 10 years of age, children do not show significant connectivity at rest but do show positive connectivity during emotion processing (Gee et al., 2013; Gabard-Durnam et al., 2014). However, these samples contained few young children (i.e., 4-6 years) and did not directly compare connectivity across contexts within the same children. Thus, open questions remain regarding development of amygdala-mpfc circuitry, including 1) are amygdala and mpfc functionally connected at rest before 10 years? 2) how does context modulate amygdala-mpfc connectivity and age-related changes within this network? 3) are individual differences in amygdala-mpfc connectivity behaviorally-relevant? To address these questions, we collected functional MRI data from 30 four- and 36 six-year-old children and 19 adults both at rest and while passively viewing a movie portraying rich social-emotional interactions. The final sample of children included 19 four year olds and 24 six year olds who completed both rest and movie scans with minimal motion artifact. Children also completed the Simplified Eyes Reading Test (SERT) in which they inferred a person's mental state based on their eyes (Peterson & Slaughter, 2009). Whole-brain analyses using a bilateral amygdala seed region demonstrated significant connectivity with MPFC, OFC, insular, and temporal regions in children and adults for both rest and movie scans. Children, but not adults, showed amygdala connectivity with posterior superior temporal (STG) and posterior cingulate (PCC). These findings suggest that, counter to previous suggestions, amygdala and mpfc are functionally connected at rest even in young children. Connectivity across contexts was compared using regions-of-interest created from the WFU pickatlas (normalized to a pediatric template) to include amygdala, MPFC, OFC, INS, STG and PCC. While these regions did not reveal age-related changes in amygdala connectivity, MPFC and OFC did show significantly greater amygdala connectivity during movie viewing than rest. Furthermore, performance on the SERT was significantly related to connectivity between the amygdala and OFC during the movie, but not rest, conditions. These findings reveal connectivity between amygdala and mpfc is present early, context-dependent, and behaviorally-relevant.

Disclosures: **E. Redcay:** None. **K. Rice:** None. **T. Riggins:** None.

Nanosymposium

775. Long-Term Memory: Medial Temporal Lobe

Location: 143A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 775.08

Topic: F.01. Human Cognition and Behavior

Support: NIMH MH060668

Title: Changes in resting-state connectivity during the consolidation of delay and trace fear conditioning memory

Authors: *D. H. SCHULTZ[1], N. L. BALDERSTON[1], L. S. HOPKINS[1], F. J. HELMSTETTER[1,2];
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Abstract: Resting-state functional connectivity (RSFC) is fairly stable across time, but changes can be observed in clinical populations or after performance of behavioral tasks. We hypothesized that changes in RSFC could be used to examine the time course of memory consolidation. Consolidation refers to the process of stabilizing a memory after initial learning. Previous work has suggested that activity and plasticity are required in different brain regions at different times during consolidation. We used delay and trace fear conditioning as models of emotional memory. In both types of conditioning, a previously neutral visual stimulus is paired with an aversive outcome. The brain circuitry supporting delay conditioning is well characterized, with the amygdala being a critical component. Trace conditioning also relies on the amygdala, but a temporal gap between the stimuli recruits activity in several other brain areas including the hippocampus and medial prefrontal cortex. On the first day of the experiment we collected an 8 minute baseline resting-state scan. After that scan participants were randomly assigned to one of three groups. One group underwent delay fear conditioning, the second group underwent trace fear conditioning, and a third group was exposed to the same stimuli in an explicitly unpaired manner and served as a control group. After training all participants received a second resting-state scan. Twenty-four hours later as well as one week following the conditioning session the participants reported back to the scanner and for another resting-state scan. A memory test was conducted one week after training. We identified several regions of interest and extracted the low frequency component of the BOLD signal from each of these regions at each time point for each participant. These correlation values were compared across groups for each of the resting-state scans. RSFC was increased between the amygdala and several other regions for both the delay and the trace group at twenty-four hours after acquisition. We found increased RSFC for the trace group relative to the delay group in several brain regions thought to support trace conditioning. The most robust differences in RSFC were apparent twenty-four hours following acquisition and most of those increases had diminished by seven days after conditioning. Altered RSFC following conditioning can be observed for several hours and these changes may reflect processes related to memory consolidation. These results could be used to inform targets for therapeutic intervention in anxiety disorders.

Disclosures: D.H. Schultz: None. **N.L. Balderston:** None. **L.S. Hopkins:** None. **F.J. Helmstetter:** None.

Nanosymposium

775. Long-Term Memory: Medial Temporal Lobe

Location: 143A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 775.09

Topic: F.01. Human Cognition and Behavior

Title: Functional connectivity differences of the anterior and posterior entorhinal cortex in humans

Authors: *S. ANTERAPER[1], S. WHITFIELD-GABRIELI[2], M. R. GEDDES[2], C. TRIANTAFYLLOU[3], J. GABRIELI[2], A. T. MATTFELD[2];
[2]Dept. of Brain and Cognitive Sci., [1]MIT, Cambridge, MA; [3]Dept. of Radiology, Massachusetts Gen. Hosp., Charlestown, MA

Abstract: The entorhinal cortex (ERC) is a key region of the medial temporal lobe (MTL) memory system functioning as the main bridge between the hippocampal formation and neocortex. Histological studies in nonhuman primates have identified largely segregated patterns of connectivity between the perirhinal cortex (PRC) and anterior ERC and the parahippocampal cortex (PHC) and posterior ERC. This model, in humans would be translated as functional similarities between PRC and anterior ERC, and PHC and posterior ERC. However, functional connectivity differences between sub-regions within ERC have yet to be reported in humans. Functional connectivity MRI (fcMRI) of MTL sub-regions has remained challenging. The MTL is susceptible to field-inhomogeneity related artifacts that worsen with thick slices that are typically employed for whole brain imaging. Higher spatial resolutions are key to exploring this region but usually at the cost of whole brain coverage and reduced signal-to-noise ratio (SNR). Using a combination of high resolution and whole-brain coverage, our aim was to elucidate the differences in resting state fcMRI from anterior and posterior ERC. Multichannel brain array coil was employed for this purpose to compensate for the trade off in signal-to-noise ratio that occurs with small voxel volumes. Healthy participants (N=51) were imaged using a 3T Siemens Tim Trio using the 32 Channel coil. High-resolution (2 mm3) resting-state scans were preprocessed using standard procedures in SPM8, followed by functional connectivity analyses using CONN toolbox. Source regions of interest (ROIs) for the anterior and posterior ERC were chosen as Brodmann areas (BAs) 34 and 28 respectively. BAs 35 and 36 served as source ROIs for PRC and PHC. When comparing anterior ERC connectivity to posterior ERC connectivity, the cortical topography was remarkably similar to that observed when comparing PRC connectivity to PHC connectivity. These results suggest that the functional connectivity of the anterior ERC with the neocortex is more similar to the PRC, while the posterior ERC connectivity with the cortex

shares similarities to the PHC. Although anatomically adjacent, the striking differences in functional connectivity as revealed in this study may untangle our current understanding of MTL pathologies in the ERC such as Alzheimer's disease. An important contributing factor that made the exploration of ERC possible at the whole- brain level is the 32 Channel head coil, which affords a higher spatial resolution without the usual reduction in temporal SNR.

Disclosures: S. Anteraper: None. **A.T. Mattfeld:** None. **S. Whitfield-Gabrieli:** None. **J. Gabrieli:** None. **C. Triantafyllou:** None. **M.R. Geddes:** None.

Nanosymposium

775. Long-Term Memory: Medial Temporal Lobe

Location: 143A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 775.10

Topic: F.01. Human Cognition and Behavior

Support: Medical Research Service of the Department of Veterans Affairs

NIMH Grant MH24600

Title: Medial temporal lobe activity distinguishes veridical from illusory recognition memory

Authors: *Z. J. URGOLITES[1,4], C. N. SMITH[1,4], L. R. SQUIRE[1,4,2,3];
[1]Psychiatry, [2]Neurosciences, [3]Psychology, UCSD, San Diego, CA; [4]Veterans Affairs San Diego Healthcare Syst., San Diego, CA

Abstract: The hippocampus is important for forming long-term memory. Some studies have found that the hippocampus is activated to a similar degree for hits (true memories) and false alarms (false memories) (e.g., Cabeza et al., 2001). Those studies used lures that were closely related to studied items (e.g., lures that belonged to the same semantic categories as targets) as well as foils that were unrelated to studied items (e.g., foils that belonged to different categories than targets). This manipulation may serve to increase the similarity in brain activity for true and false memories. We asked participants to make old/new recognition memory judgments using confidence ratings (1-6 scale) for 240 previously studied scenes and 240 new scenes during fMRI scanning. Confidence ratings were higher for hits than for false alarms (5.5 vs. 4.6). Comparisons between hits and false alarms revealed that activity in the hippocampus and parahippocampal gyrus was significantly higher for hits (true memories) than for false alarms (false memories). However, when hits and false alarms were selected so as to equate confidence

ratings, no medial temporal lobe (MTL) activity was observed. That is, higher MTL activity for hits than for false alarms was driven by the higher confidence ratings associated with hits. In addition, in a separate analysis, MTL activity was positively related to confidence ratings for targets but was not related to confidence ratings for foils. Together, the findings suggest that MTL is sensitive to memory strength and that the higher confidence levels associated with hits makes it possible for the MTL to distinguish true memories from false memories.

Disclosures: Z.J. Urgolites: None. **C.N. Smith:** None. **L.R. Squire:** None.

Nanosymposium

775. Long-Term Memory: Medial Temporal Lobe

Location: 143A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 775.11

Topic: F.01. Human Cognition and Behavior

Support: FP7-PEOPLE-MC-ITN-2008-n 238157-EBRAMUS

"LaCaixa" foundation fellowship (2013)

Title: Singing in the brain: Neural correlates of auditory binding of lyrics and melodies

Authors: *I. ALONSO[1,2,3], R. VALABRÈGUE[2], S. DUPONT[4,3], S. SAMSON[1,4];
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Abstract: Songs naturally bind lyrics and melody into a unified representation. Nevertheless, although learning and remembering songs is rather common, the cerebral bases underlying such auditory binding have received little attention. To address binding of lyrics and melodies for song encoding, we compared BOLD signal changes during subsequent encoding of songs after unified presentation of songs (passive condition) and non-unified presentation, where lyrics and melody were presented separately (active condition). In both conditions participants were instructed to generate mental images of the songs and to repeat them covertly until the next trial. BOLD signal during encoding was parametrically modulated by a subsequent memory index based in the accuracy and confidence in the later recognition test. We expected passive condition to recruit cortical areas in the temporal lobe, as well as the parahippocampal and the prefrontal cortex. Based on the higher cognitive demands for binding non-unified information, we

hypothesize active condition to require greater activation of several brain areas including the hippocampus and the inferior parietal lobe, as opposed to passive binding. The main effect of successful encoding in the passive condition recruited areas of the left hemisphere including the middle temporal gyrus, the inferior frontal gyrus and the postcentral gyrus. Successful encoding of the active condition revealed activation in the left middle temporal gyrus, in addition several regions bilaterally including the superior frontal gyrus, the cerebellum, the caudate and the anterior cingulate gyrus. In line with our predictions a comparison between active against passive condition revealed greater activity in the right hippocampus. Furthermore, other regions including the left middle temporal gyrus, the left insula, the left anterior cingulate gyrus, the left parahippoampal gyrus, the right inferior frontal gyrus, the right fusiform gyrus and the cerebrellum bilaterally were also shown in this contrast. This study supports and discusses the role of medial temporal lobe in the binding of lyrics and melodies in songs. However, in light of these results, other structures such as basal ganglia, the cerebellum and the angular gyrus seem to be important for auditory binding and need further investigation.

Disclosures: I. Alonso: None. **R. Valabrègue:** None. **S. Dupont:** None. **S. Samson:** None.

Nanosymposium

775. Long-Term Memory: Medial Temporal Lobe

Location: 143A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 775.12

Topic: F.01. Human Cognition and Behavior

Support: Swiss National Science Foundation, Grant no.320030-132447

Title: Rapid memory consolidation by transient theta oscillations between the human medial temporal lobe and the rest of the brain

Authors: *R. THÉZÉ, A. G. GUGGISBERG, L. NAHUM, A. SCHNIDER;
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Abstract: Improved retention of items repeated after presentation of interfering items is known as the Spacing Effect (SE) and is considered a ubiquitous phenomenon. In an earlier study (James et al. Hippocampus 2009; 19: 371-8), we found that immediate picture repetition induced a positive frontal potential at 200-300 ms emanating from the left medial temporal lobe (MTL), possibly indicating the interference with an ongoing consolidation process. In the present study, we investigated functional connectivity changes associated with immediate repetition. We

obtained high-density electroencephalographic (EEG) recordings from 14 healthy subjects during a continuous recognition task in which pictures were either repeated immediately or after 9 intervening items (spaced repetition). The procedure induced the expected SE: 30 minutes later, stimuli that had been repeated after 9 intervening items were better recognized than immediately repeated stimuli. Waveform EEG analysis of the initial learning run again revealed a positive frontal potential emanating from left MTL at 200-300 ms in response to immediately repeated stimuli. Coherence analysis was performed over 1000 ms after stimulus presentation to detect brain areas changing their connectivity with the rest of the brain in a frequency band between 3 and 45 Hz. It revealed that immediately repeated stimuli induced distinct connectivity changes at 200-400 ms: coherence specifically increased in the theta band (3-7.5 Hz) between the parahippocampal region -left more that right- and the rest of the brain. This increase was absent during spaced repetition. The coherence change varied between subjects: it was stronger in subjects who had better long-term retention of immediately repeated stimuli and, thus, a weak SE. These findings suggest that transient theta-band synchronization between the MTL and the rest of the brain at 200-400 ms reflects a memory stabilization signal that protects against immediate repetitions' interference with ongoing consolidation. The SE appears to reflect an advantage of off-line consolidation in subjects lacking transient theta oscillations upon immediate repetition.

Disclosures: R. Thézé: None. **A.G. Guggisberg:** None. **L. Nahum:** None. **A. Schnider:** None.

Nanosymposium

776. Predictive Coding: Human Cognition

Location: 152A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 776.01

Topic: F.01. Human Cognition and Behavior

Support: Marie Curie International Outgoing Fellowship

ONR N00014-13-1-0672

MH103814

Title: Hierarchical detection of auditory regularities: Cortical layers and cortical hierarchies suggest a predictive coding mechanism

Authors: *L. MELLONI[1,2,3], T. THESEN[3], C. M. SCHWIEDRZIK[4], W. DOYLE[3], O. DEVINSKY[3], D. FRIEDMAN[3], P. DUGAN[3], I. ULBERT[5], S. S. CASH[6], W. SINGER[2,7], C. E. SCHROEDER[1,8], E. HALGREN[9];
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Abstract: One of the most pervasive types of short term plasticity is repetition suppression (RS). This reduction of the neural response to repetition of the same stimulus is thought to increase sensitivity to new information. A signature of this sensitivity to differences is the mismatch negativity (MMN), which reflects the ability to perform comparisons between consecutive stimuli and to respond to a violation of regular input streams. It is unclear whether the MMN reflects an automatic consequence of stimulus-specific adaptation (SSA), an active comparison process between a sensory memory and the current stimulus, or a combination of both as proposed by Predictive Coding (PC). To test these conflicting accounts we combine large-scale ECOG recordings with investigations of the laminar generators of the MMN in human cortex during an oddball paradigm. The last stimulus in a sequence of 4 syllables was either a standard or a deviant that was either hard or easily distinguished from the standard. We recorded ECOG during task performance in 5 epilepsy patients undergoing invasive monitoring for seizure foci. 4 patients were also implanted with linear array electrodes, providing simultaneous recordings from all layers in several temporal lobe sites. We observed RS in the high gamma band (50-150Hz) in a localized network around the superior temporal gyrus (STG) and (pre)motor cortex. The degree of RS in posterior STG predicted whether a subject would perceive a minimally deviant stimulus as same or different from the standard. When focusing on the oddball stimuli we identified two distinct types of responses, interdigitated across recording sites: One that showed SSA to standard stimuli, along with an oddball response, and another that did not respond to the standard stimuli, but responded exclusively to the deviant stimulus. As these latter sites did not show any responses to standard stimuli, SSA does not appear to be a factor in the neuronal operation that they index. Instead, the findings point to a comparison process that integrates over auditory events and signals deviation from an established pattern. Supporting this hypothesis, we could predict from these electrodes whether a difficult deviant would be perceived as different from the standard or not, although the acoustics were always the same. At the laminar scale, CSD analysis revealed that deviant stimuli elicited increased transmembrane current flow in supragranular layers. This is in line with the hypothesis that prediction errors are most evident supragranular layers. These findings suggest that the MMN arises from SSA and an active comparison process between a learned regularity and the deviant, consistent with PC.

Disclosures: **L. Melloni:** None. **T. Thesen:** None. **C.M. Schwiedrzik:** None. **W. Doyle:** None. **O. Devinsky:** None. **D. Friedman:** None. **P. Dugan:** None. **I. Ulbert:** None. **S.S. Cash:** None. **W. Singer:** None. **C.E. Schroeder:** None. **E. Halgren:** None.

Nanosymposium

776. Predictive Coding: Human Cognition

Location: 152A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 776.02

Topic: F.01. Human Cognition and Behavior

Title: Abstract encoding of auditory sequences and deviance detection in the macaque brain

Authors: ***L. WANG**[1], L. UHRIG[2], B. JARRAYA[2], S. DEHAENE[2];
[1]Inst. of Cognitive Neurosci., Shanghai, China; [2]INSERM-CEA Cognitive Neuroimaging Unit, Paris, France

Abstract: The predictive coding hypothesis postulates that, at multiple levels of processing, the brain actively predicts forthcoming sensory inputs rather than passively registering them. In humans, hierarchical predictive coding models have been supported by studies of auditory sequence processing. However, neural evidence in support of this model in non-human primates remains scarce. Using fMRI, we investigated the encoding of auditory regularities in awake monkeys. To ensure stimulus novelty, auditory paradigms were presented to monkeys only during the fMRI session and not during the training sessions. In a first experiment (Uhrig et al., J. Neuroscience, 2014), we used the local- global paradigm (Bekinschtein et al, PNAS, 2009) to study the encoding of auditory sequences at two hierarchical levels: local detection of rare tones and global detection of rare sequences. While first-order (local) sequence violations activated the auditory pathway, second-order (global) violations,activated a prefronto-parietal and cingulate cortical network, a 'macaque' homologue of the global neuronal workspace. In a second experiment, we examined whether monkeys encode such auditory sequences at an abstract level. Does the monkey brain memorize specific auditory sequences, or is it able to extract the abstract pattern underlying several auditory sequences? In each fMRI block, we presented multiple auditory sequences that shared a systematic "algebraic" pattern, for instance "xxxY" (three identical sounds followed by a different one). Variations in the temporal spacing and frequency of the individual tones ensured that only the abstract syntactic and numerical structure was predictable. We then tested for brain responses to novel sequences that either respected the original pattern, or violated it, either by changing the number of items or the "syntax" of the

sequence (e.g. going from xxxY to xxxx). Using such abstract rules, fMRI activation to deviant sequences was displaced beyond the auditory cortex. Numerical deviants specifically activated the intraparietal cortex, while both numerical and syntactic deviants activated the ventral prefrontal cortex. Human fMRI data, collected in the same paradigm, allowed us to study the monkey-human homologies and differences in these areas. Altogether, our fMRI studies demonstrate that monkeys can represent the abstract structure of auditory sequences, use it to predict future auditory events, and detect violations of these predictions at several distinct cortical levels.

Disclosures: **L. Wang:** None. **L. Uhrig:** None. **B. Jarraya:** None. **S. Dehaene:** None.

Nanosymposium

776. Predictive Coding: Human Cognition

Location: 152A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 776.03

Topic: F.01. Human Cognition and Behavior

Support: Wellcome Trust Grant 091593/Z/10/Z

DFG Fellowship AU 423/1-1

Title: Modulation of sensory predictions by temporal attention: A DCM/MEG study

Authors: *R. AUKSZTULEWICZ[1], K. FRISTON[2];
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Abstract: Although attention and expectation affect behaviour in a similar manner (improving stimulus detection and recognition), their effects on early evoked neural activity are antagonistic. Specifically, event-related responses are enhanced by attention and attenuated by expectation. These opposing effects have been reconciled in the predictive coding framework, according to which unexpected stimuli yield large prediction errors that are propagated forward in the processing hierarchy. Crucially, these prediction errors are subject to attentional modulation, equivalent to being weighted by their precision. The aim of the present study was to test the predictive coding account of attention and sensory expectation using magnetoencephalographic data and computational modelling (dynamic causal modelling; DCM). Temporal attention and sensory expectation were orthogonally manipulated in an auditory mismatch paradigm. Brief auditory tones (sine waves at 6 possible frequencies) were played in a roving oddball sequence

over the course of multiple trials. In any given trial, tones could be presented at two latencies with 50% probability, independent for each latency. Healthy volunteers (N=20, 10 female) were instructed to attend to one of the latencies (randomised across blocks) and respond to tone omissions. Analysis of event-related field (ERF) amplitude revealed opposing effects of sensory expectation and temporal attention. Crucially, mismatch responses (ERF difference waves between frequency deviants and standards, reflecting sensory expectation violation) were enhanced by temporal attention, speaking against the supposedly pre-attentive nature of mismatch detection. The antagonistic effects of attention and expectation were modelled using DCM based upon a canonical microcircuit. Several alternative models, allowing for extrinsic vs. intrinsic connectivity modulations at different levels of processing hierarchy from sensory to frontal areas, were compared using Bayesian model selection. The winning model explained mismatch responses in terms of recursive interplay of sensory predictions and prediction errors. On the other hand, temporal attention was associated with state-dependent changes in gain of inhibitory interneurons in primary auditory cortices, extending previous experimental and theoretical work on the role of precision-weighted prediction errors in perception.

Disclosures: **R. Auksztulewicz:** None. **K. Friston:** None.

Nanosymposium

776. Predictive Coding: Human Cognition

Location: 152A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 776.04

Topic: F.01. Human Cognition and Behavior

Support: ERC grant FP7-Predispike

JSMF award #220020335/UHC

ANR-10-LABX-0087 IEC

ANR-10-IDEX-0001-02 PSL

Title: Experimental evidence for circular inference in schizophrenia: Beyond predictive coding?

Authors: ***S. DENEVE**, R. JARDRI;
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Abstract: Recent experimental and computational studies suggest a subtle impairments of excitatory-to-inhibitory (E/I) balance or regulation in many neurological and psychiatric conditions, including schizophrenia. Considering that the brain constructs hierarchical causal models of the external world, we showed previously that failure to maintain the E/I balance in a neural hierarchy will result in pathological "Circular belief propagation": bottom-up and/or top-down messages will be reverberated, misinterpreting prior beliefs as sensory evidence and vice-versa. As a result, sensory and/or prior information are counted multiple times instead of (as normally) once. Circular inference explains the emergence of erroneous percepts, the patient's general overconfidence, the learning of "unshakable" but erroneous causal relationships and a paradoxical immunity to perceptual illusions. This "belief propagation" framework can be understood as a form of "predictive coding", but applied to probabilities rather than variables. However, in addition to top down expectations being subtracted from bottom-up sensory evidence, sensory evidence are also subtracted from top-down expectations. This introduces two types of "inhibitory" control loops with very distinct roles, probable anatomical basis and associated pathologies. In order to test the model quantitatively, we tested schizophrenic patients and control subjects on a simple Bayesian reasoning task. Subject had to report their confidence in the provenance of a single sample (a "black" fish), based on the proportion of such fishes in two lakes, and on prior information. We found that patients greatly over-estimate the strength of sensory evidence, but do not over-estimate the prior. Despite being extremely variable from patient to patient, the behavior is very well fitted on a subject-per-subject basis by a parametric circular inference model, where ascending loops (those preventing sensory evidence from being reverberated as top down predictions) are impaired compare to controls. The level of impairment is strongly correlated with severity along positive and dis-organizational dimensions, but not the "negative" dimension (PANNS scoring system). This suggests that hallucinations, delusions and disorganized thoughts are paradoxically linked to high-level over-interpretation of sensory observations, which are given too much credit through reverberation, rather than on a dominance of prior beliefs or impaired prediction errors.

Disclosures: S. Deneve: None. **R. Jardri:** None.

Nanosymposium

776. Predictive Coding: Human Cognition

Location: 152A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 776.05

Topic: F.01. Human Cognition and Behavior

Support: NSF Graduate Research Fellowship 338 2009090358

NIH Grant EY013588

NSF Grant BCS-0727115

NSF Grant BCS-1228535

Title: Modeling the emergence of gamma band oscillations in the visual thalamocortical pathway

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Abstract: Gamma oscillations have been hypothesized to play a central role in active vision, contributing to inter-areal information selection and routing. Furthermore, emerging evidence suggests that gamma oscillations play a fundamental role in feedforward neuronal communication. In order to constrain these hypotheses, it is useful to know what the "minimal" circuitry for gamma is in the cortex, and under what conditions gamma is prominent during active vision. We recently showed (Bastos et al., 2014) that gamma oscillations are an emergent property of the cortex, and are not present in the LGN of alert behaving monkeys. Here, we set out to explain these results in terms of an underlying bio-physically realistic network model composed of Hodgkin-Huxley neurons. The prime candidate mechanism to explain our experimental results was recurrent inhibition: the anatomy of the cortex is full of recurrence, allowing excited pyramidal cells to activate inhibitory cells, which can then inhibit a large number of pyramidal cells and set the pace of the gamma oscillation (e.g., the classical "PING" - Pyramidal Interneuron Network Gamma rhythm). However, this recurrence, of excitation back onto local inhibitory cells, appears to be lacking within the primate LGN (but perhaps not in the feline LGN) at an anatomical level. In our model of the LGN lacking local recurrence, the circuit did not engage in gamma oscillations even when the Thalamic Reticular Nucleus (TRN), a structure that provides inhibition onto relay cells and is excited by geniculocortical collaterals, was included. However, the cortex, which did contain recurrent inhibition, entered a rhythmic mode despite receiving arrhythmic LGN input. Intriguingly, even when the cortex projected back onto the LGN in a gamma rhythmic mode, this was not sufficient to entrain the LGN at gamma. In our model of the feline LGN, which did include local recurrent inhibition, gamma oscillations did emerge - which may explain interspecies differences between cats and primates. We conclude that the circuitry of the primate LGN is well-suited to ignore cortical gamma rhythms, to focus on a sparse and temporally precise neuronal code, and to maximize the feedforward throughput of information from the retina to the cortex. Finally, we also modeled recently published results (Bastos et al., 2014) showing that despite no gamma band coherence between

the LGN and V1, the two structures did engage in synchronization at lower (alpha and beta) frequencies, with directionality analysis revealing that feedforward communication occurred at beta and feedback communication in alpha. We discuss these results in light of predictive coding models of brain function.

Disclosures: A. Bastos: None. **H.B.S. Esmeraldo:** None. **G.R. Mangun:** None. **W.M. Usrey:** None.

Nanosymposium

776. Predictive Coding: Human Cognition

Location: 152A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 776.06

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant 1U54MH091657

Title: Human visual areas exert feedforward and feedback influences through distinct frequency channels

Authors: *G. MICHALAREAS[1], J. VEZOLI[1], S. VAN PELT[1], H. KENNEDY[2], P. FRIES[1]; [1]Fries Lab., Ernst Strüngmann Inst. (ESI) For Neurosci., Frankfurt Am Main, Germany; [2]Stem Cell and Brain Res. Institute, INSERM U846, Lyon, France

Abstract: The communication between brain areas is subserved by rhythmic neuronal synchronization. Invasive animal studies have found inter-areal rhythmic synchronization to be particularly prominent in the alpha/beta and the gamma band. In visual cortex, local gamma-band synchronization predominates in superficial layers, and alpha/beta band synchronization in deep layers. Layer-wise anatomical connections differentiate between feedforward and feedback directions: The feedforward (feedback) output of an area originates primarily in superficial (deep) layers, and this preference is stronger for projections traversing more hierarchical levels. Therefore, we investigated whether feedforward and feedback signalling are subserved by distinct frequency bands. To do this for the human brain, we recorded 36 subjects with MEG, source-projected the signals and calculated Granger-causal (GC) influences between 7 visual areas, for which clear homologue areas exist in the non-human primate brain: V1, V2, V4, MT, PIT (TEO), V7 (DP) and pIPL (7A). The seven areas resulted in GC influences for 21 pairs of areas. For the same area pairs, we obtained retrograde anatomical tracing data, quantifying the degree to which a given inter-areal projection was feedforward or feedback. GC influences were

determined for frequencies between 1 and 140 Hz. For each frequency separately, we correlated the respective GC influences with the tracing data, across area pairs and subjects. This correlation revealed two significant frequency bands corresponding closely to the typical alpha/beta and gamma bands. The sign of the correlation demonstrated that inter-areal gamma-band synchronization subserves feedforward communication, and inter-areal alpha/beta-band synchronization subserves feedback communication. In anatomy, the pattern of feedforward and feedback connections across all pairs of visual areas is largely consistent with a global hierarchy, assigning each area to a particular level. We had found GC influences to be correlated with anatomical feedforward/feedback relations. Therefore, we tested whether the pattern of GC influences across all 21 area pairs was also consistent with a global hierarchy, i.e. a functional hierarchy based purely on GC influences. A hierarchy based on gamma-band GC influences correlated significantly with the most recent anatomical hierarchy (R=0.942, P=0.0015). A beta-based hierarchy showed a lower correlation (R=0.66, P=0.0609). These results suggest that feedforward and feedback communication in the visual system uses separate frequency channels.

Disclosures: G. Michalareas: None. **J. Vezoli:** None. **S. Van Pelt:** None. **H. Kennedy:** None. **P. Fries:** None.

Nanosymposium

776. Predictive Coding: Human Cognition

Location: 152A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 776.07

Topic: F.01. Human Cognition and Behavior

Support: ERC StG 2012_311751 - Brain reading of contextual feedback and predictions

BBSRC BB/G005044/1 - Visual Prediction

Title: Predictive coding of auditory and contextual information in early visual cortex - evidence from layer specific fMRI brain reading

Authors: *L. MUCKLI;
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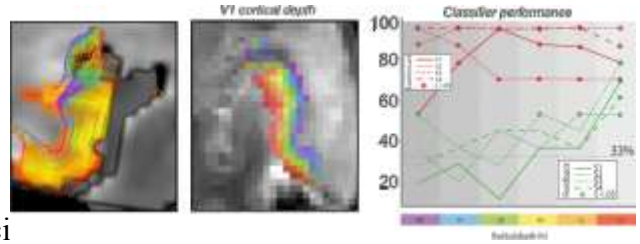
Abstract: David Mumford (1991) proposed a role for reciprocal topographic cortical pathways in which higher areas send abstract predictions of the world to lower cortical areas. At lower cortical areas, top-down predictions are then compared to the incoming sensory stimulation. One

question that arises within this framework is the following: Do descending predictions remain abstract, or do they translate into concrete level predictions, the ‘language’ of lower visual areas? We have exploited a strategy in which feedforward information is occluded in parts of visual cortex: i.e. along the non-stimulated apparent motion path, behind a white square that we used to occlude natural visual scenes, or by blindfolding our subjects (Muckli & Petro 2013). By presenting visual illusions, contextual scene information or by playing sounds we were able to capture feedback signals within the occluded areas of the visual cortex. Multi-voxel pattern analysis (MVPA) of the feedback signal reveals that they are more abstract than the feedforward signal. Using high resolution functional brain imaging (BOLD fMRI, GE-EPI 0.8 mm) we found that feedback is sent to the outer cortical layers of V1. We further show that feedback to V1 can originate from auditory information processing (Vetter, Smith & Muckli 2014). Auditory induced feedback is especially strong in the periphery of V1 and contains categorical abstract information. I would like to argue that these feedback signals function as abstract signals, i.e. priors in a Bayesian framework, biasing future processing at the earliest cortical stage of V1. References: Mumford (1991) On the computational architecture of the neocortex - the role of the thalamocortical loop. Biol Cybernetics Muckli & Petro (2013) Network interactions: non-geniculate input to V1. Curr Opin Neurobiol. Vetter, Smith & Muckli (2014) Decoding Sound and Imagery Content in Early Visual Cortex. Current Biology Clark (2013) Whatever Next? Predictive Brains, Situated Agents, and the Future of Cognitive Science. Behav Brain Sci



Disclosures: L. Muckli: None

Nanosymposium

776. Predictive Coding: Human Cognition

Location: 152A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 776.08

Topic: F.01. Human Cognition and Behavior

Support: ERC grant 260347

CNRS

Title: The contribution of frequency-specific activity to hierarchical information processing in human auditory cortices

Authors: *A.-L. GIRAUD[1], L. FONTOLAN[1], C. LIEGEOIS-CHAUVEL[2], B. MORILLON[3]; [1]Dept. d'Etudes Cognitives, Dept of Neurosci. - Univ. of Geneva, Geneva, Switzerland; [2]3INSERM U1106 – Inst. de Neurosciences des Systèmes, Marseille, France; [3]Dept. of Psychiatry, Columbia Univ., New-York, NY

Abstract: That feed-forward and top-down propagation of sensory information use distinct frequency bands is an appealing assumption for which evidence remains scarce. Using human depth recordings from two auditory cortical regions in both hemispheres, while subjects listened to sentences, we show that information traveled in each direction using separate broad frequency channels. Bottom-up and top-down propagation dominated in the gamma and the theta/beta band respectively. The predominance of low frequency (theta/beta) activity for top-down information transfer was confirmed by cross-regional frequency nesting, which indicates that the power of gamma activity in A1 was modulated along the phase of theta/beta activity sampled from AAC. This nesting effect was absent in the opposite direction. Finally, we show that information transfer does not proceed continuously, but rather by time windows where bottom-up or top-down processing dominates. These findings suggest that the brain uses both frequency- and time-division multiplexing to optimize directional information transfer.

Disclosures: **A. Giraud:** None. **L. Fontolan:** None. **B. Morillon:** None. **C. liegeois-chauvel:** None.

Nanosymposium

776. Predictive Coding: Human Cognition

Location: 152A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 776.09

Topic: F.01. Human Cognition and Behavior

Support: JSPS Postdoctoral Fellowships for Research Abroad (H23)

CIHR

Title: Coexistence of representations of basic tastes and hedonic valence in subregions of gustatory cortices

Authors: *J. CHIKAZOE[1], D. H. LEE[2], N. KRIEGESKORTE[3], A. K. ANDERSON[1]; [1]Dept. of Human Develop., Cornell Univ., Ithaca, NY; [2]Dept. of Psychology, Univ. of Toronto, Toronto, ON, Canada; [3]Med. Res. Council, Cognition and Brain Sci. Unit, Cambridge, United Kingdom

Abstract: Taste is critical to survival since it allows discrimination of beneficial and harmful food, based on subjective hedonic criteria. Rodent and monkey studies have demonstrated that gustatory information is processed in the insula, frontal operculum and orbitofrontal cortex. Human fMRI studies also revealed activation in these gustatory cortices evoked by taste perception. On the other hand, these gustatory cortices are also known to code valence information which make it complicated to interpret the results of these studies. Given the close relationship between basic tastes and hedonic valence, it is possible that exactly the same subregion in the gustatory cortices may code both basic tastes and valence. However, this still remains unclear since previous studies rarely investigated both basic taste discrimination and valence representations in a single study. In the present fMRI study, we employed a novel approach to disentangle components of valence and basic taste representations, based on representational similarity. We first modeled each trial as a separate event, then computed similarity of activation patterns in a region of interest across trials. Instead of investigating correspondence between similarity of activation patterns and similarity of respective properties (i.e. valence and basic tastes) separately, we decomposed the similarity of activation patterns into valence and basic taste components using a multiple regression model. By shifting the region of interest across a whole brain (i.e. searchlight analysis), we explored which brain regions code valence and/or basic tastes. This analysis revealed that subregions in the gustatory cortices distinguish basic tastes irrespective of valence, whereas other subregions code both valence and basic tastes. This suggests that taste quality may be experienced not only as a purely chemosensory dimension, but also as a hedonic sensory experience.

Disclosures: J. Chikazoe: None. **D.H. Lee:** None. **N. Kriegeskorte:** None. **A.K. Anderson:** None.

Nanosymposium

776. Predictive Coding: Human Cognition

Location: 152A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 776.10

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant F32MH08543301A1

NIH Grant 5R01MH075706

Title: Representation of predacity of animal species in the human brain

Authors: *A. C. CONNOLLY, J. V. HAXBY;
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Abstract: A landmark study (Rips 1973) in the semantic representation of animal categories suggested that predacity and size were central dimensions for predicting behavioral judgments of semantic similarity among animal species. While neuroimaging studies have found evidence for taxonomic representation for animals in neural activity (Connolly 2012, Sha In press), we know of no studies that show representation of predacity in the brain. In this fMRI study we locate networks that reflect predacity related neural processing independent of taxonomic class, size, and low-level visual features. Using support-vector machine pattern classification within whole-brain searchlights, we mapped networks that distinguish activation patterns elicited by viewing images of high-predacity animals (e.g., wolves, scorpions) versus low-predacity animals (e.g., rabbits, ladybugs). We controlled for taxonomic class by training and testing across class-based data folds - we trained the classifiers to distinguish between high and low predacity for one class (eg, mammals or reptiles) and tested for generalization to another class (bugs). These analyses yielded predacity-relevant networks in the right superior temporal sulcus (RSTS), left anterior intraparietal sulcus (LIPSa), and calcarine sulcus. We then used clustering to group the predacity-sensitive surface nodes into separate networks based on similarity of local representational spaces. The clustering solution comprised four large clusters: CALC, LIPSa, and a division of RSTS into anterior (RSTSa) and posterior (RSTSp). Multidimensional scaling within these regions revealed the clearest distinction between high and low predacity in the RSTSa. In a complementary set of analyses, we mapped the brain for classification based on taxonomic class controlling for predacity. These analyses largely replicated our previous findings showing a separation of mammals, reptiles, and bugs along a lateral-to-medial animacy continuum that is most evident throughout ventral temporal and lateral occipital cortices. We further compared neural similarities across various brain regions with model similarity matrices for predacity, taxonomy, size, and early vision. The similarity analysis between regions and models show that RSTSa was most similar to the predacity model, followed by RSTSp, and LIPSa. The early visual regions identified in both the predacity and taxonomy analyses were closest to the early vision model, and the lateral occipitotemporal regions were most similar to the taxonomy model. In summary, these new findings suggest a distinct network spanning RSTS and LIPSa for representing the predacity of animals.

Disclosures: A.C. Connolly: None. **J.V. Haxby:** None.

Nanosymposium

776. Predictive Coding: Human Cognition

Location: 152A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 776.11

Topic: F.01. Human Cognition and Behavior

Title: A comparison of the effects of transcranial direct current stimulation and caffeine on vigilance and cognitive performance during extended wakefulness

Authors: *A. MCKINLEY[1], L. K. MCINTIRE[2], J. M. NELSON[2], C. GOODYEAR[2];
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Abstract: Background: Sleep deprivation from extended duty hours is a common complaint for many occupations. Caffeine is one of the most common countermeasures used to combat fatigue. However, the benefits of caffeine decline over time and with chronic use. Objective: Our objective was to evaluate the efficacy of anodal transcranial direct current stimulation (tDCS) applied to the pre-frontal cortex at 2 mA for 30 minutes to remediate the effects of sleep deprivation and to compare the behavioral effects of tDCS with those of caffeine. Methods: Three groups of 10 participants each received either active tDCS with placebo gum, caffeine gum with sham tDCS, or sham tDCS with placebo gum during 30 hours of extended wakefulness. Results: Our results show that tDCS prevented a decrement in vigilance and led to better subjective ratings for fatigue, drowsiness, energy, and composite mood compared to caffeine and control in sleep-deprived individuals. Both the tDCS and caffeine produced similar improvements in latencies on a short-term memory task and faster reaction times in a psychomotor task when compared to the placebo group. Interestingly, changes in accuracy for the tDCS group were not correlated to changes in mood; whereas, there was a relationship for the caffeine and sham groups. Conclusion: Our data suggests that tDCS could be a useful fatigue countermeasure and may be more beneficial than caffeine since boosts in performance and mood last several hours.

Disclosures: A. McKinley: None. **L.K. McIntire:** None. **J.M. Nelson:** None. **C. Goodyear:** None.

Nanosymposium

776. Predictive Coding: Human Cognition

Location: 152A

Time: Wednesday, November 19, 2014, 1:00 PM - 4:00 PM

Presentation Number: 776.12

Topic: F.01. Human Cognition and Behavior

Support: Mind & Life Institute Varela Award

Title: Neural correlates of mind wandering and attention during focused meditation

Authors: *W. HASENKAMP[1], C. WILSON-MENDENHALL[2], L. BARSALOU[3];
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Abstract: This presentation describes a line of research that seeks to incorporate first-person subjective input into the analysis of meditation-related brain activity and connectivity. We delineate a basic model of naturalistic cognitive fluctuations between mind wandering and attentional states derived from the practice of focused attention meditation. This model proposes four intervals in a cognitive cycle: mind wandering, awareness of mind wandering, shifting of attention, and sustained attention. Using fMRI, we developed a paradigm to leverage subjective reports of awareness of mind wandering during focused attention meditation, using these reports to drive data analysis. Results revealed activity in brain regions associated with the default mode during mind wandering, and in salience network regions during awareness of mind wandering. Elements of the executive network were active during shifting and sustained attention. Further, participants with more meditation experience exhibited increased resting state functional connectivity within attentional networks, as well as between attentional regions and medial frontal regions. These neural relationships may be involved in the development of cognitive skills, such as maintaining attention and disengaging from distraction, that are often reported with meditation practice. These findings will be placed in the context of our larger understanding of identified brain networks, and future directions and applications will be discussed.

Disclosures: W. Hasenkamp: None. **C. Wilson-Mendenhall:** None. **L. Barsalou:** None.